Introduction to Radiobiology
Lesson 3

Master of Advanced Studies in Medical Physics
A.Y. 2022-23

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Human chromosomes

A ***chromosome*** is a microscopic, threadlike part of a cell that carries hereditary information in the form of genes.

Every species has a characteristic number of chromosomes; humans have 23 pairs (22 pairs are non-sex chromosomes and 1 pair is sex chromosome).

A ***gene*** is a unit of heredity that occupies a fixed position on a chromosome.

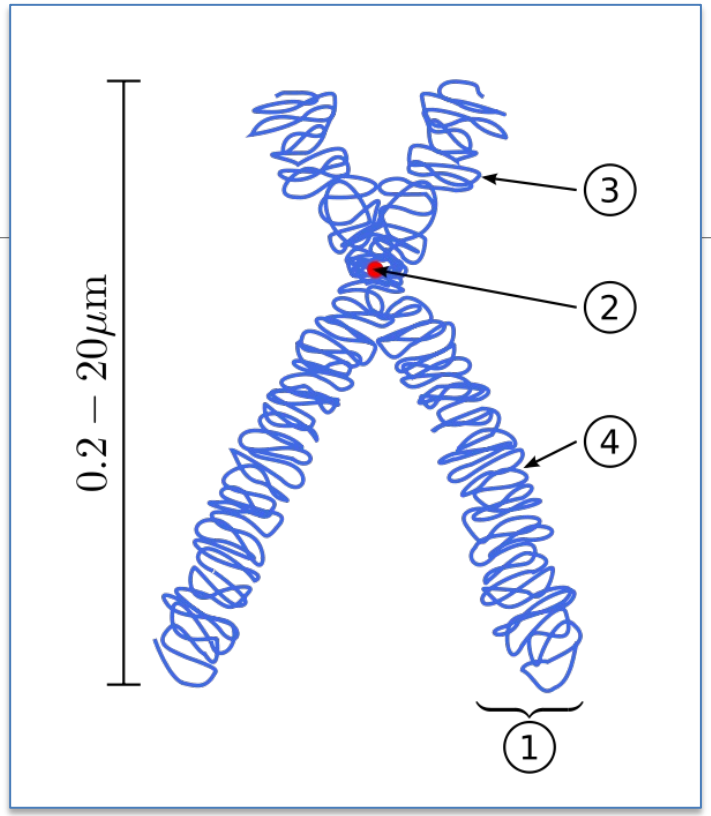
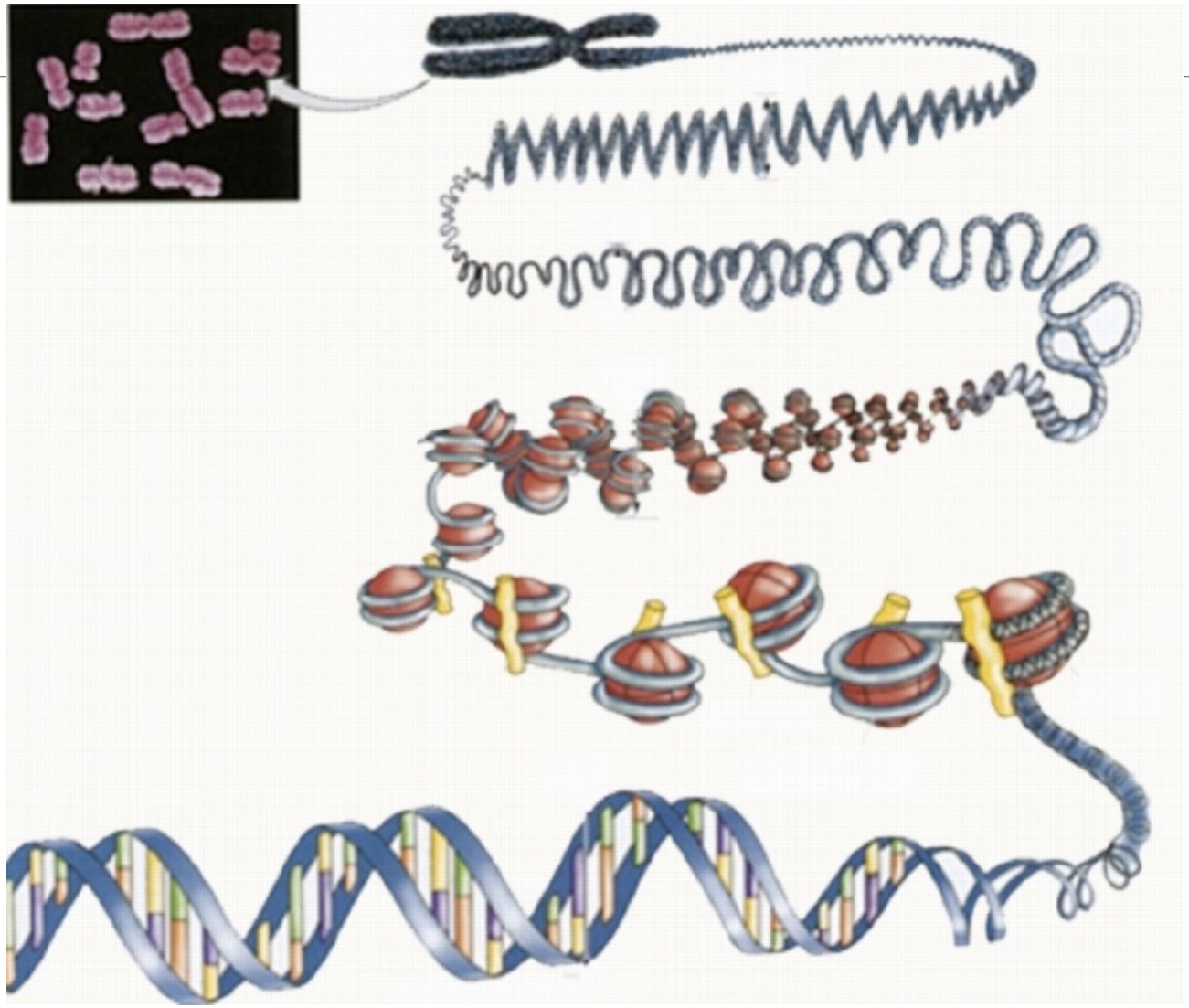


Diagram of a replicated and condensed metaphase eukaryotic chromosome.

(1) Chromatid – one of the two identical parts of the chromosome after S phase.
(2) Centromere – the point where the two chromatids touch.
(3) Short arm.
(4) Long arm.

Chromosomes are an efficient packing of DNA



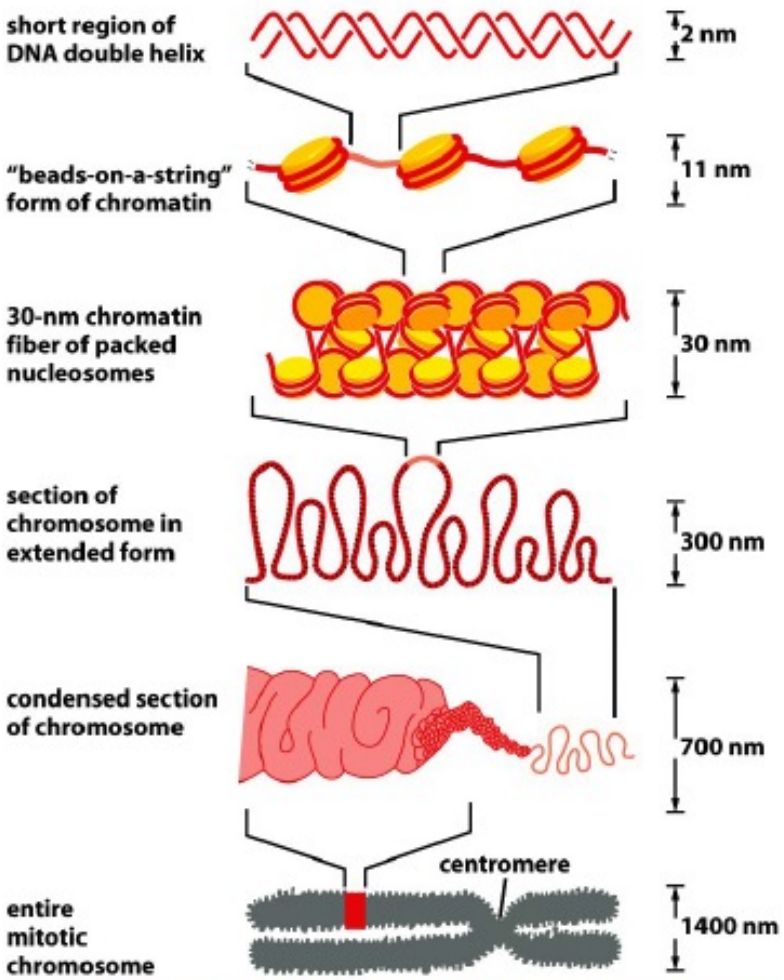
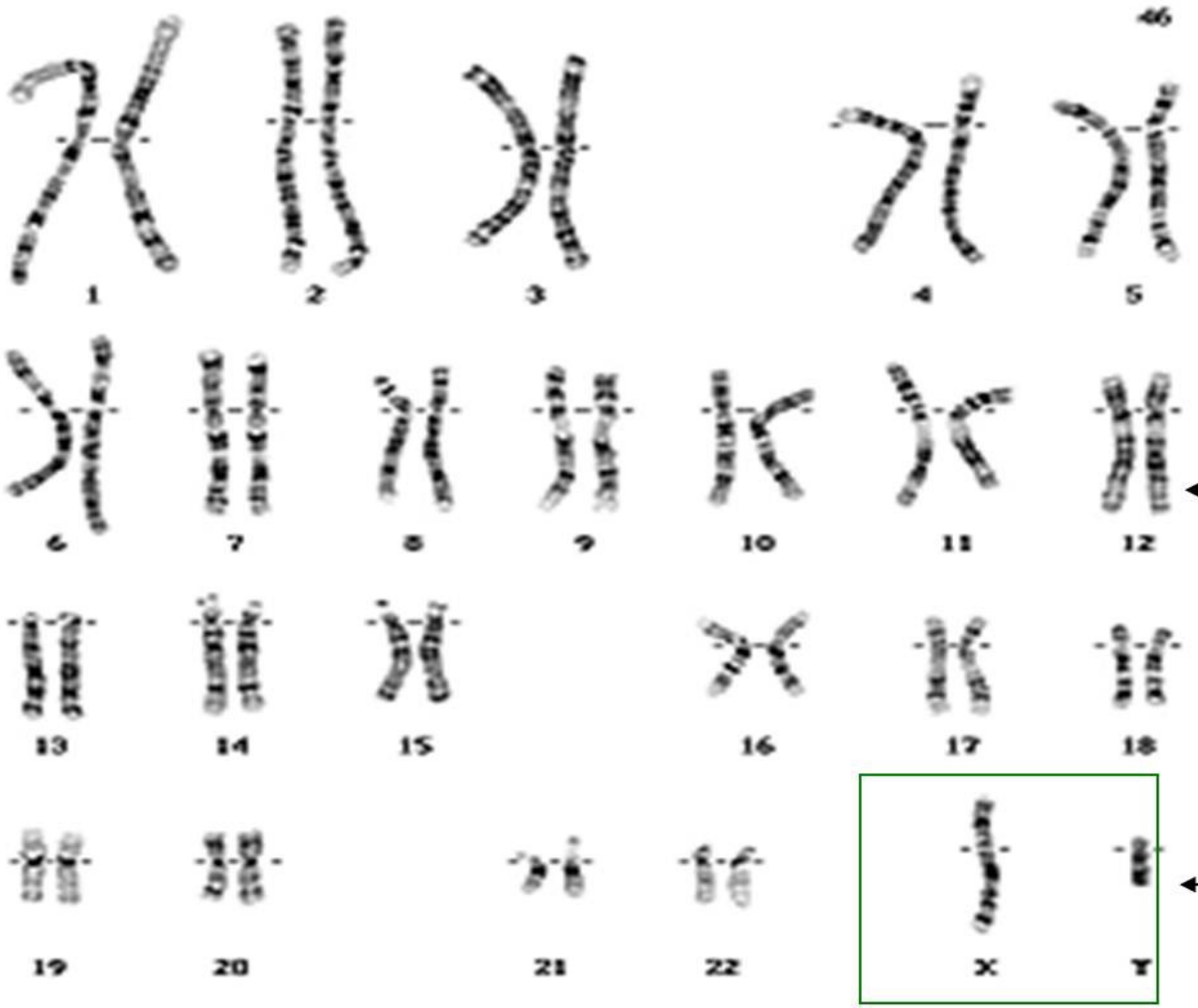


Figure 4-72 *Molecular Biology of the Cell* (© Garland Science 2008)

There are 23 chromosome pairs (46 chromosomes in all) in this karyogram of the human chromosomes



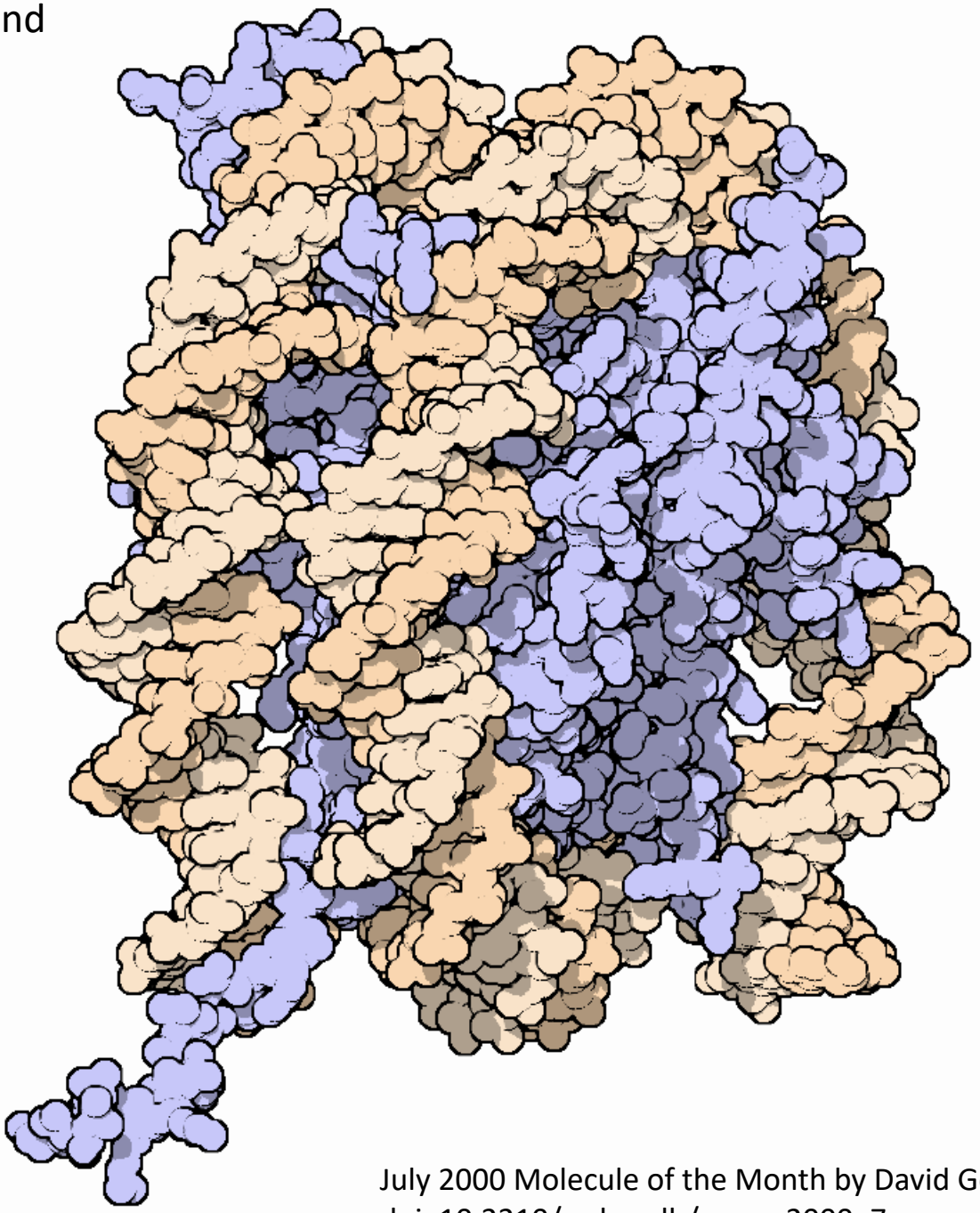
22 pairs Autosomes

1 pair sex chromosomes

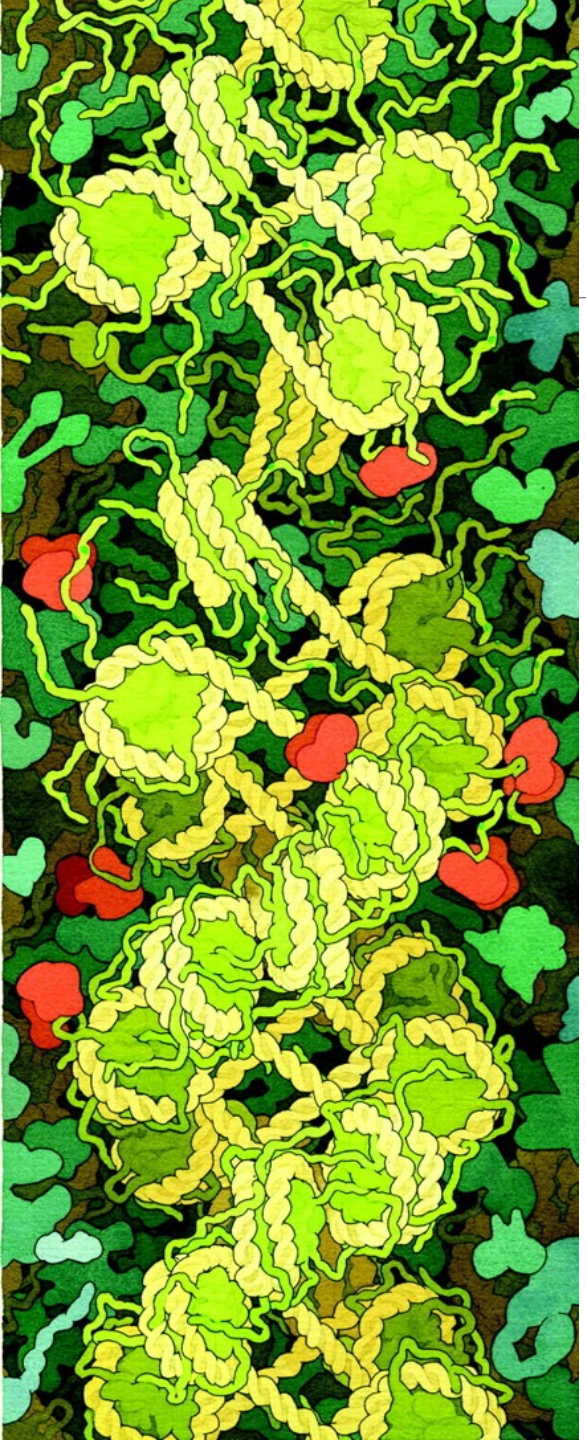
females: two X cromosomes
males: one X and one Y chromosome

DNA wrapped around
a histone molecule

(Nucleosome)



July 2000 Molecule of the Month by David Goodsell
doi: 10.2210/rcsb_pdb/mom_2000_7



A strand of chromatin is shown, with the DNA (in yellow) wrapped around the **histones** (in green).

Each little bundle of eight histones with two loops of DNA is termed a nucleosome.

At the top, the tails of the histones are acetylated at many sites (small green dots), leading to an open, accessible form of chromatin.

At the center, histone deacetylase (in red) is removing the acetyl groups.

At the bottom, the deacetylated histone tails associate with neighboring nucleosomes to form a compact, inaccessible form of chromatin.

(adapted from D. Goodsell: "The Molecular Perspective: Histone Deacetylase", *The Oncologist* **8** (2003) 389)

building block of DNA

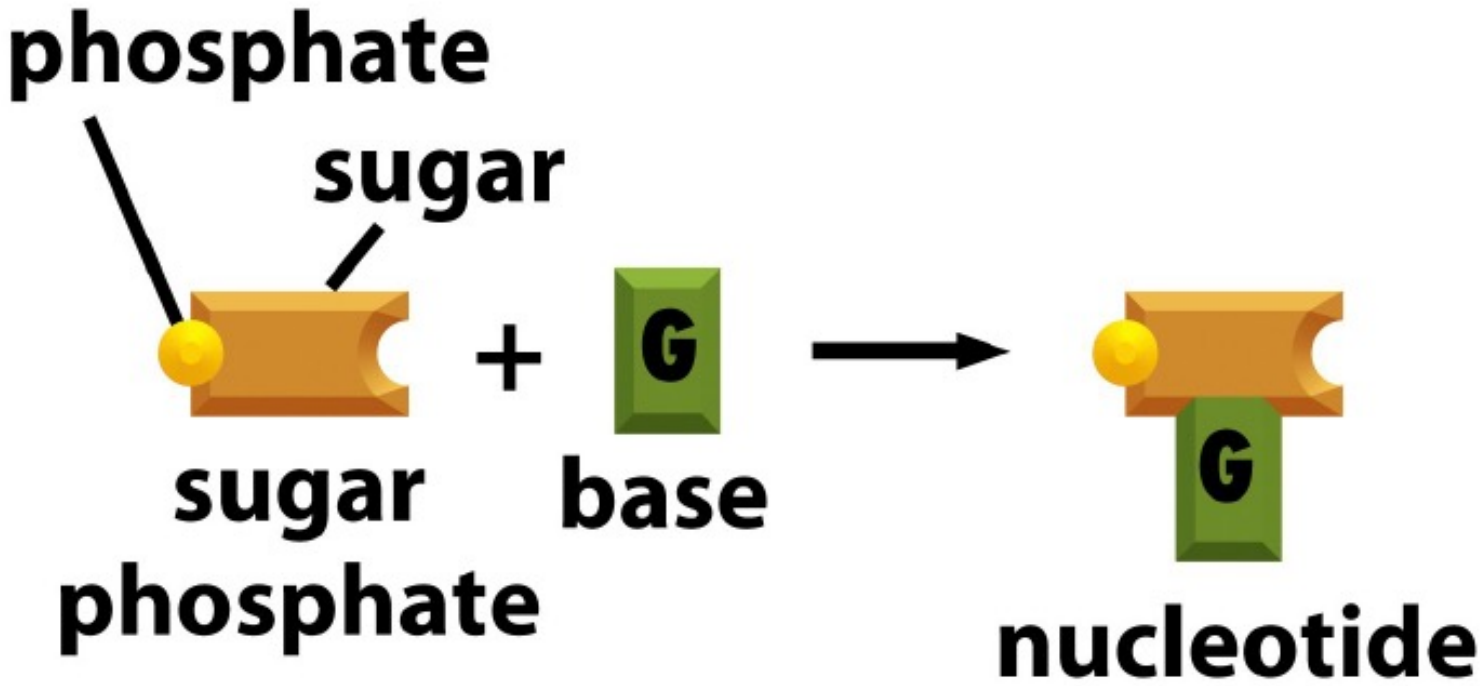
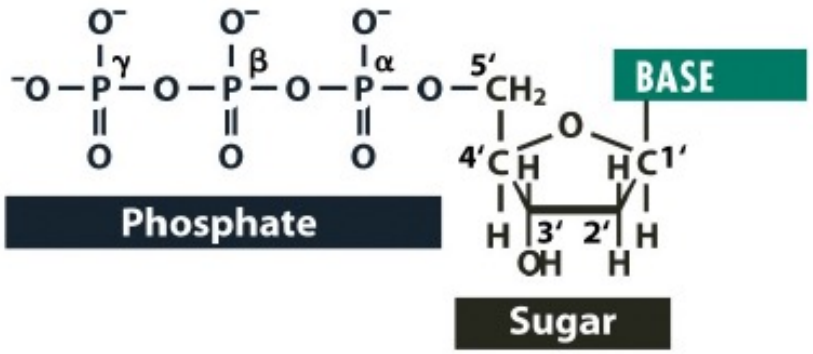


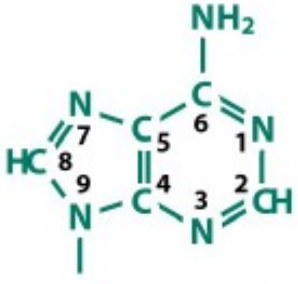
Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Nucleotides, building blocks of DNA (and RNA)

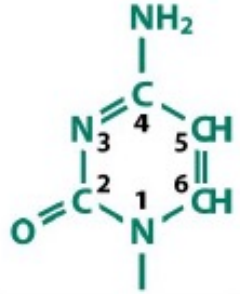
(A) A nucleotide



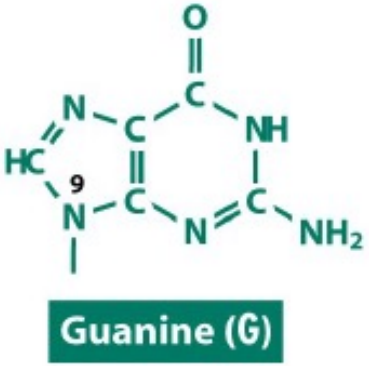
(B) The four bases in DNA



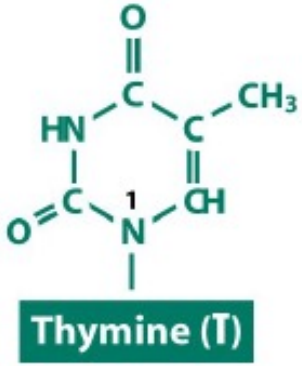
purine



pyrimidine



purine



pyrimidine

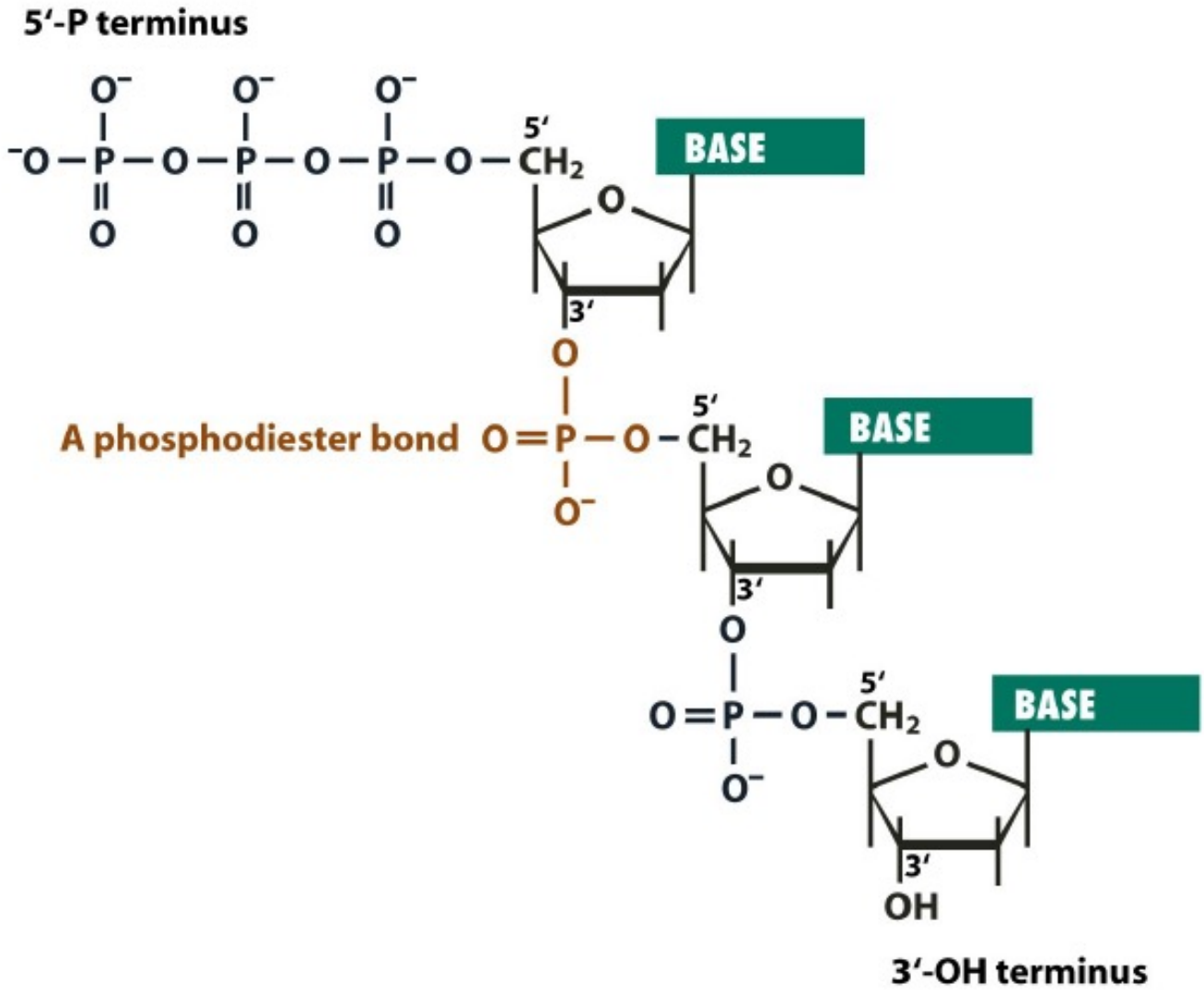


Figure 1.5 *Genomes 3* (© Garland Science 2007)

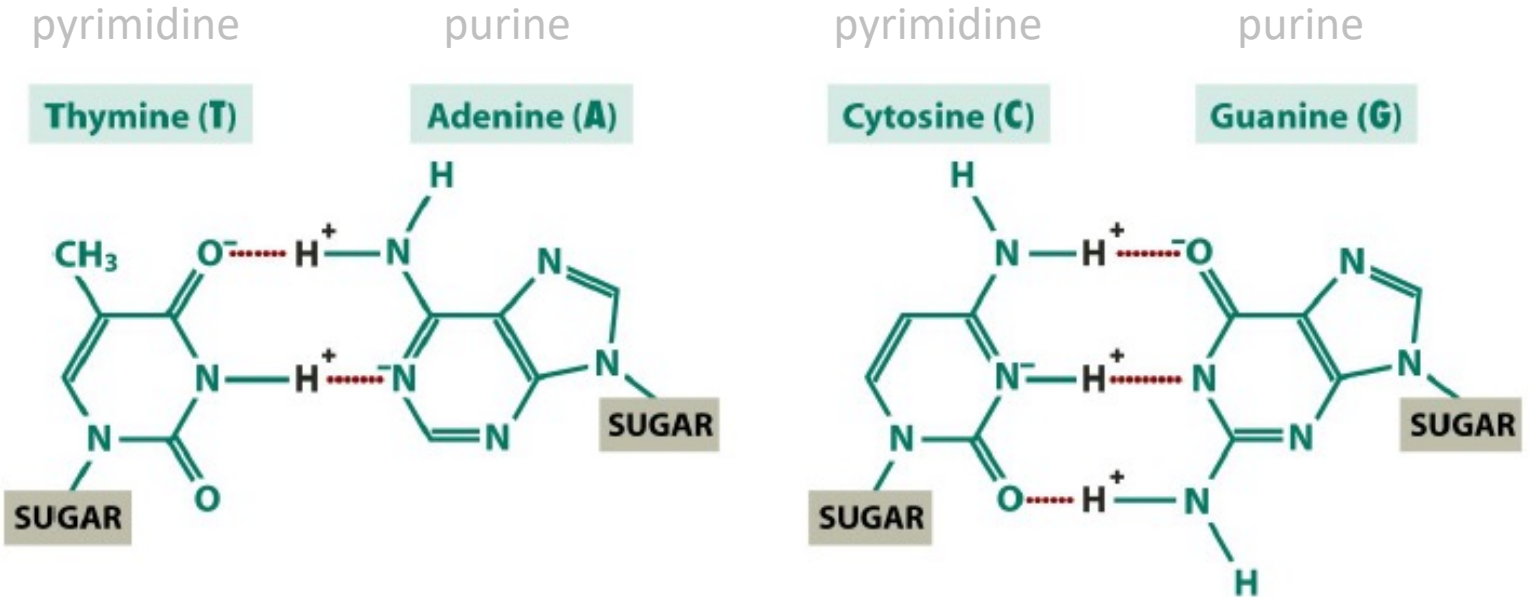


Figure 1.8b *Genomes 3* (© Garland Science 2007)

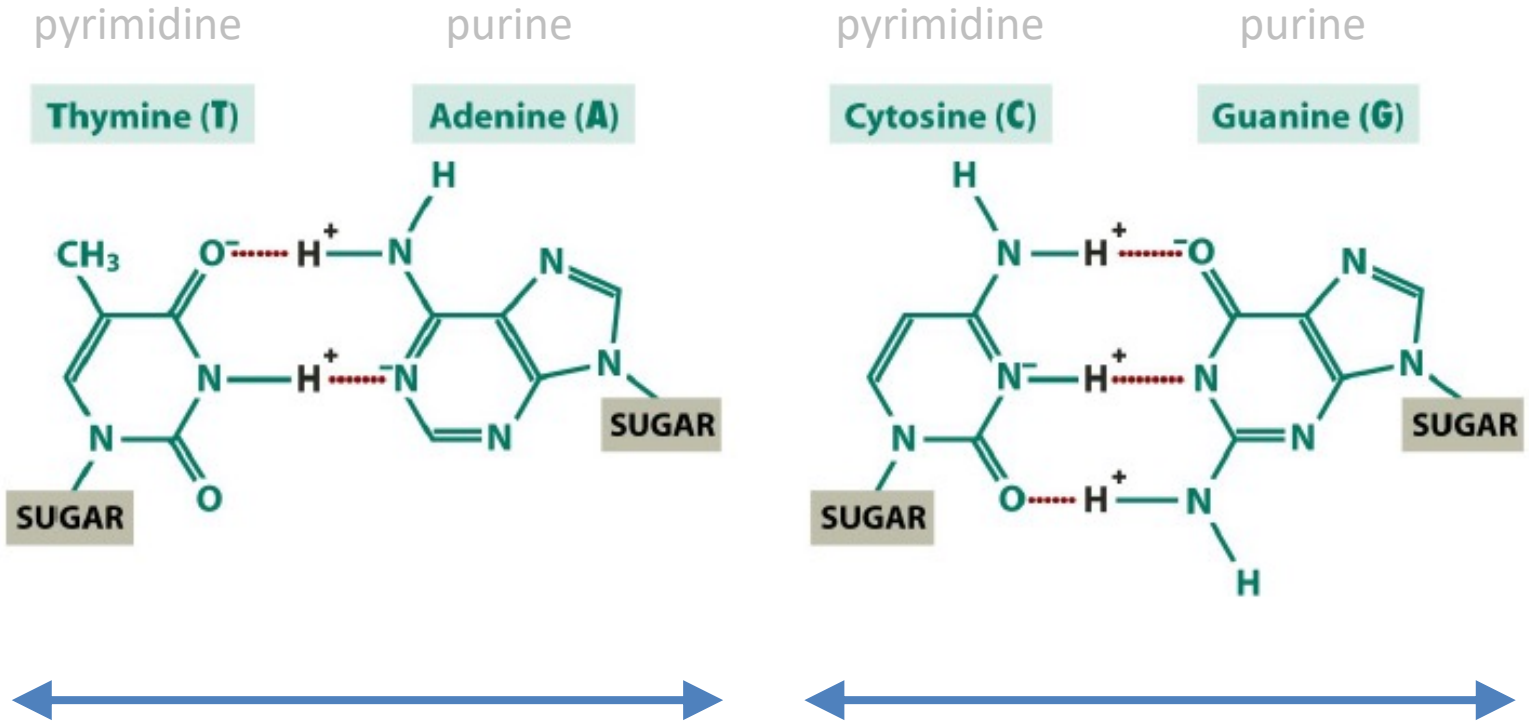


Figure 1.8b *Genomes 3* (© Garland Science 2007)

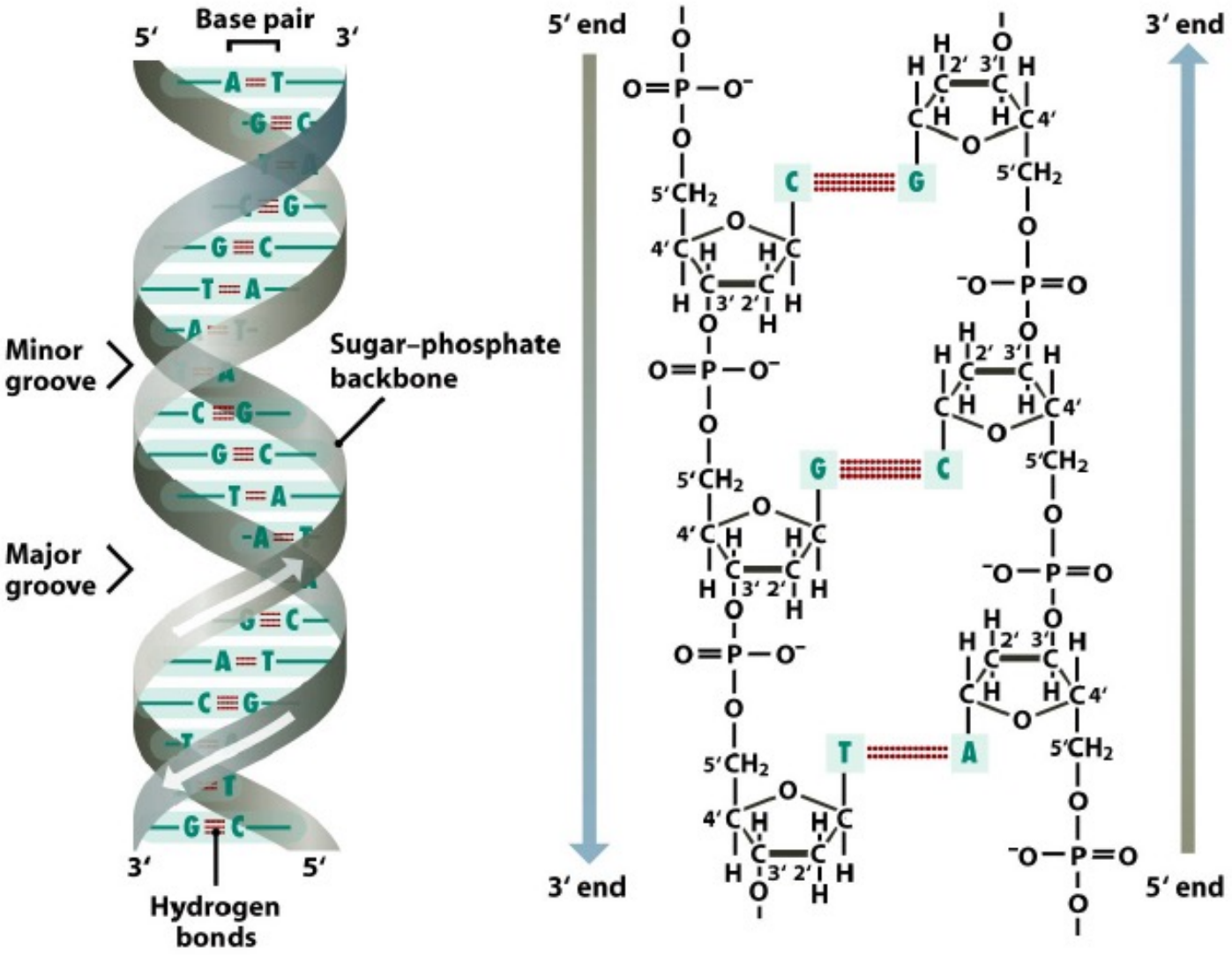


Figure 1.8a *Genomes 3* (© Garland Science 2007)

Figure 1-2b *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

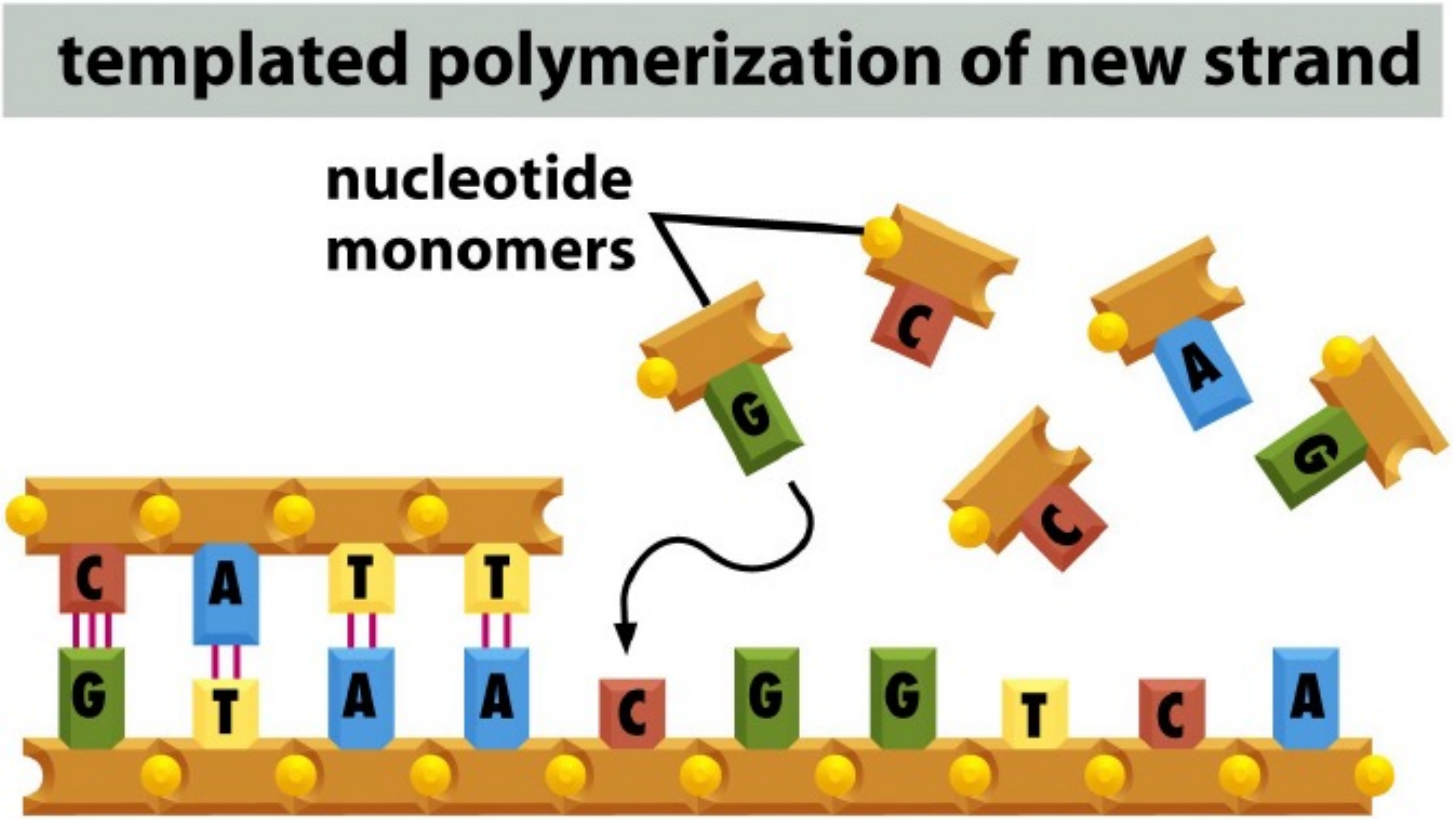


Figure 1-2c *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

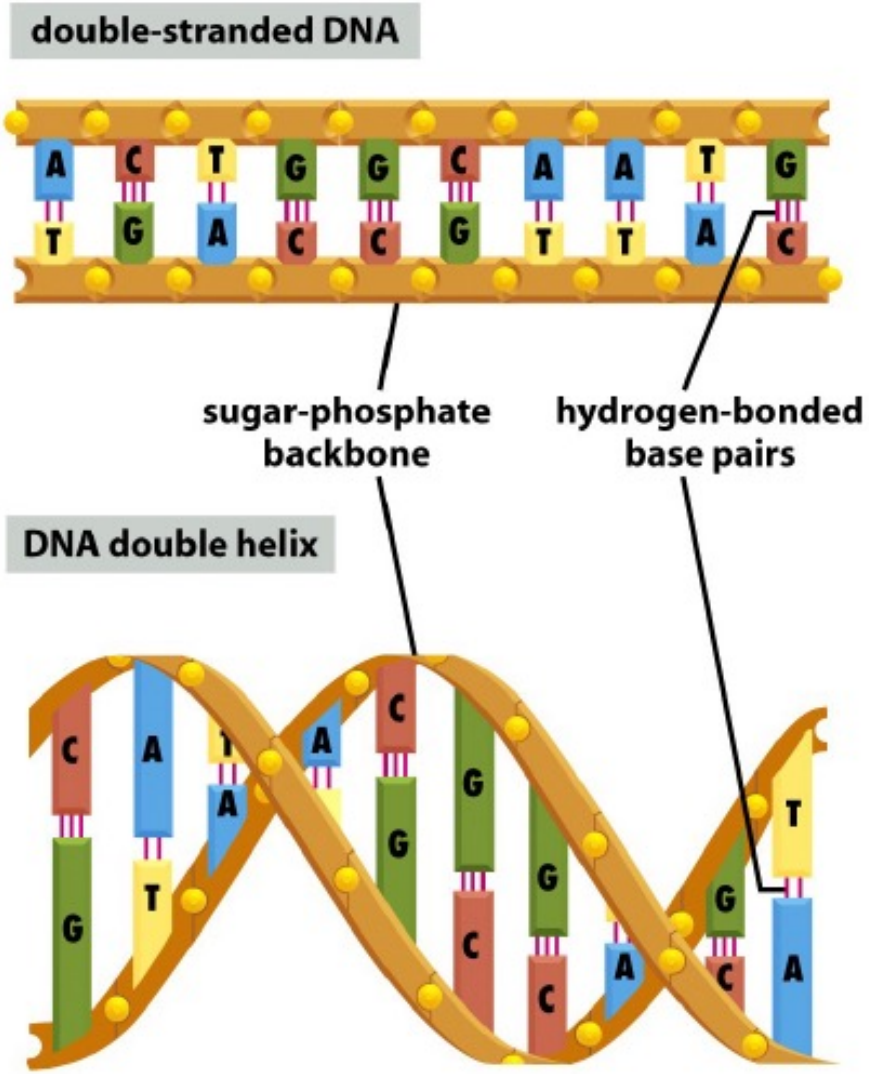


Figure 1-2d,e *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

The sequence of base pairs in DNA encodes information that leads to protein synthesis
(the **central dogma of molecular biology** – Crick, 1956)

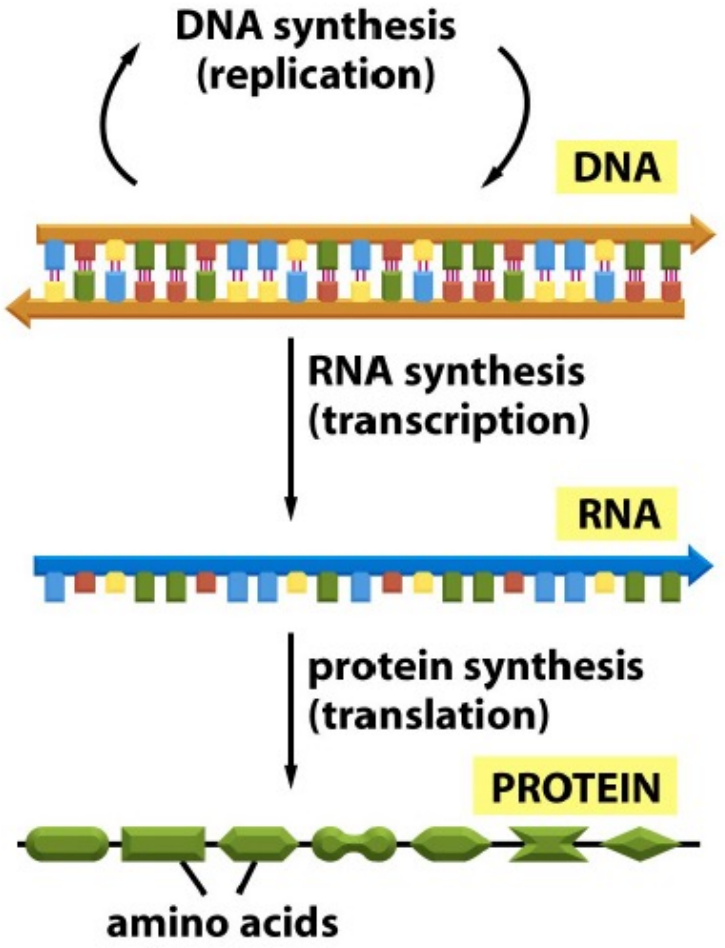


Figure 1-4 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

DNA REPLICATION or DNA DUPLICATION or DNA SYNTHESIS (synonyms) is semi-conservative

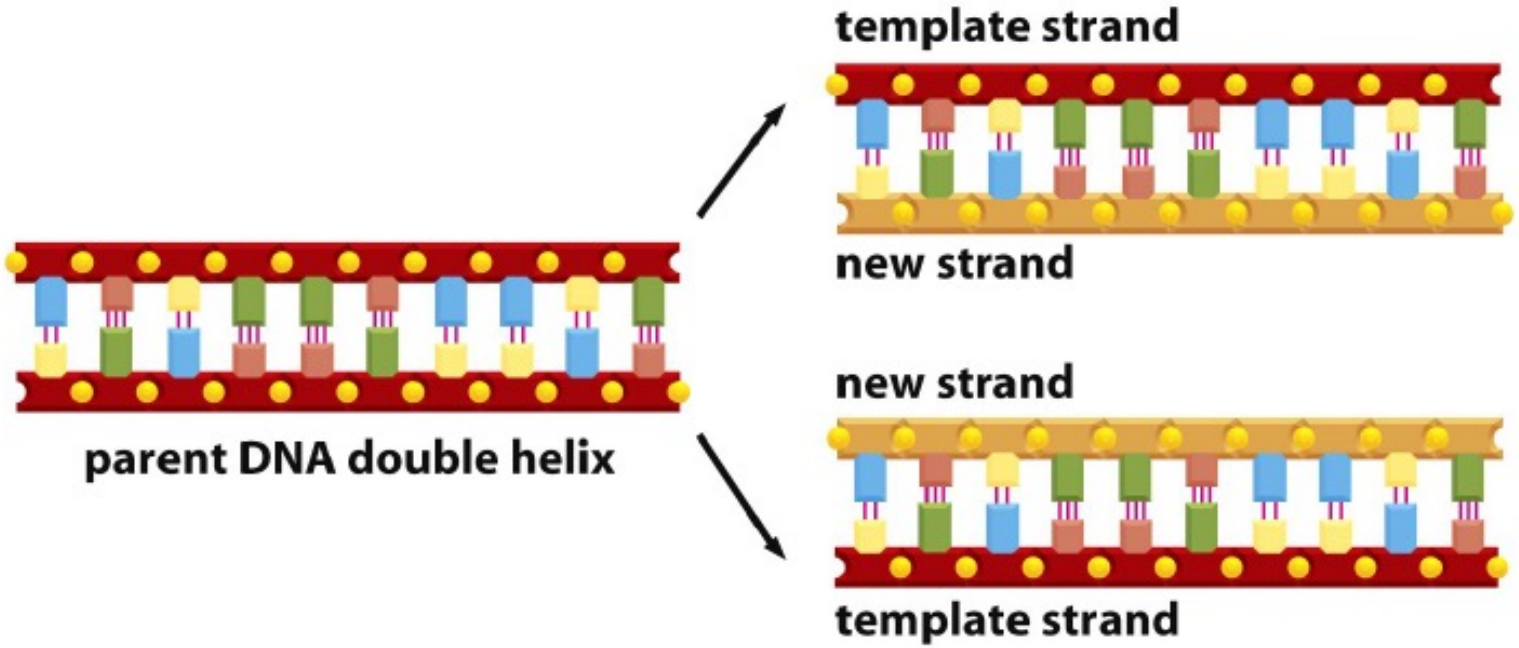
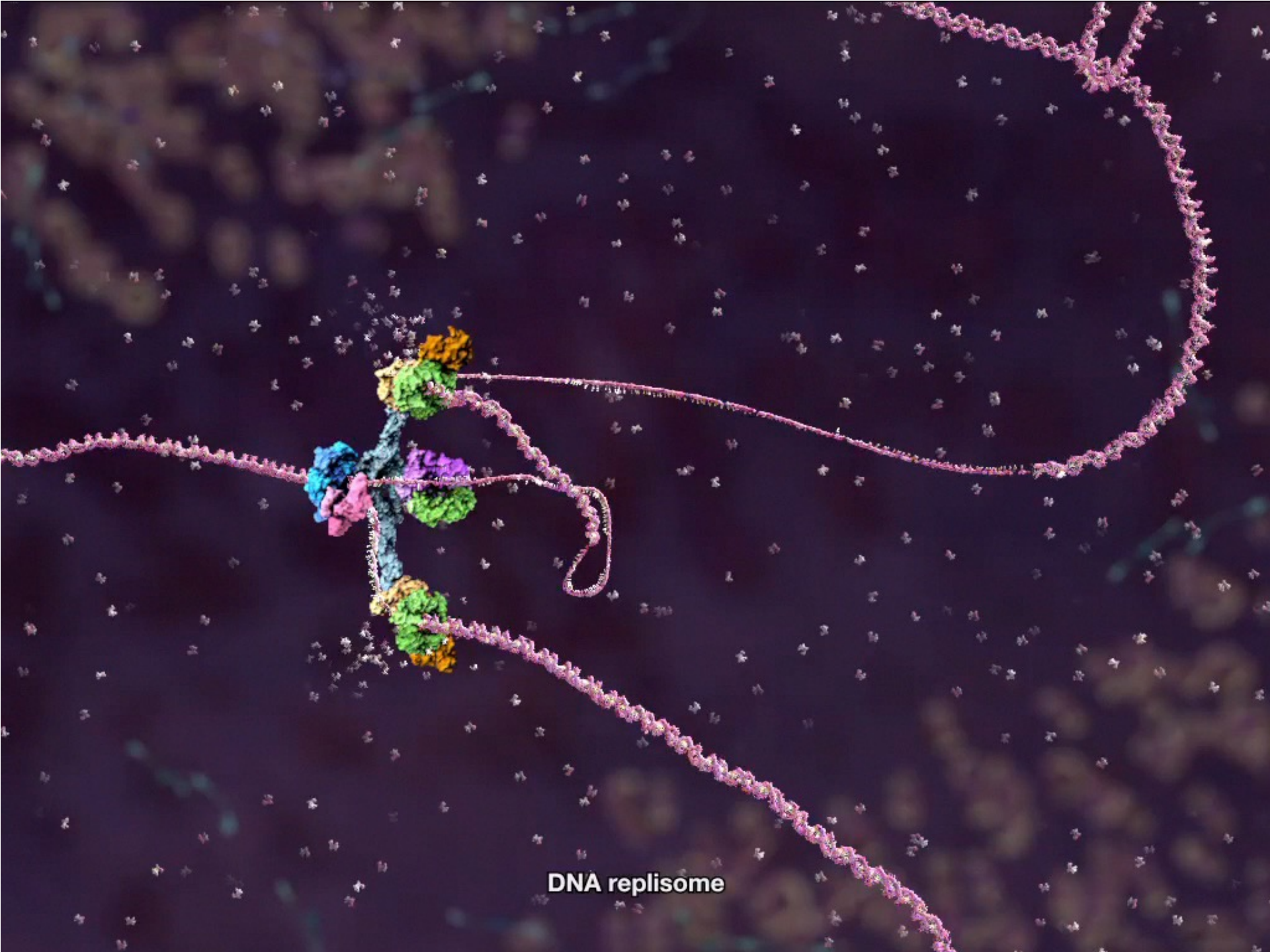


Figure 1-3 *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

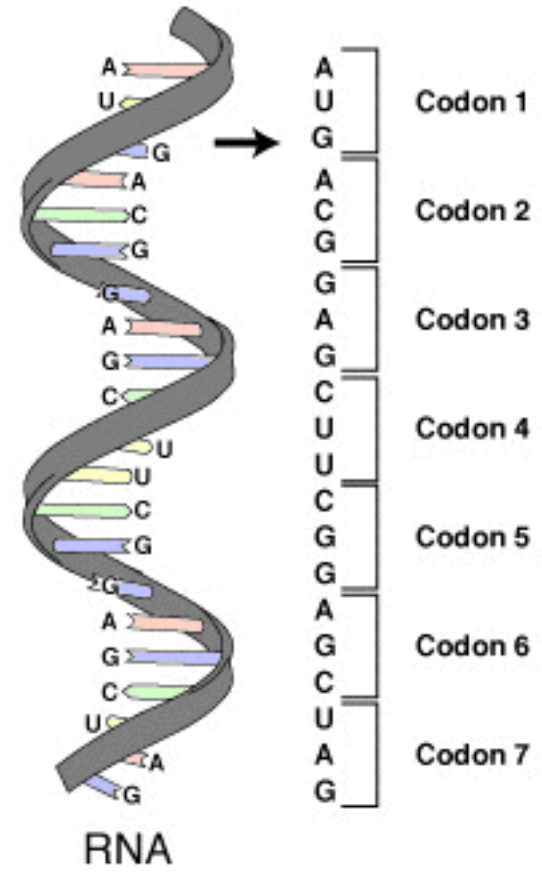

DNA replisome

The genome contains all the genetic information of the organism

Units of measurement:

bp	base pair
Kb	(10^3 bp) Kilobase
Mb	(10^6 bp) Megabase

The genetic code



- A series of codons in part of a messenger RNA (mRNA) molecule.
- Each codon consists of three nucleotides, corresponding to a single amino acid.
- The nucleotides are abbreviated with the letters A, U, G and C.
- This is mRNA, which uses U (uracil). DNA uses T (thymine) instead.
- This mRNA molecule will instruct a ribosome to synthesize a protein according to this code.

(figure and text from Wikipedia)

nonpolar | polar | basic | acidic | (stop codon)

Standard genetic code

1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	(Phe/F) Phenylalanine	TCT	(Ser/S) Serine	TAT	(Tyr/Y) Tyrosine	TGT	(Cys/C) Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine	TCA		TAA	Stop (*Ochre*)	TGA	Stop (*Opal*)	A
	TTG		TCG		TAG	Stop (*Amber*)	TGG	(Trp/W) Tryptophan	G
C	CTT		CCT	(Pro/P) Proline	CAT	(His/H) Histidine	CGT	(Arg/R) Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine	ACT	(Thr/T) Threonine	AAT	(Asn/N) Asparagine	AGT	(Ser/S) Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	ATG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine	GCT	(Ala/A) Alanine	GAT	(Asp/D) Aspartic acid	GGT	(Gly/G) Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GTG		GCG		GAG		GGG		G

Inverse table (compressed using IUPAC notation)

Amino acid	Codons	Compressed	Amino acid	Codons	Compressed
Ala/A	GCT, GCC, GCA, GCG	GCN	**Leu/L**	TTA, TTG, CTT, CTC, CTA, CTG	YTR, CTN
Arg/R	CGT, CGC, CGA, CGG, AGA, AGG	CGN, MGR	**Lys/K**	AAA, AAG	AAR
Asn/N	AAT, AAC	AAY	**Met/M**	ATG	
Asp/D	GAT, GAC	GAY	**Phe/F**	TTT, TTC	TTY
Cys/C	TGT, TGC	TGY	**Pro/P**	CCT, CCC, CCA, CCG	CCN
Gln/Q	CAA, CAG	CAR	**Ser/S**	TCT, TCC, TCA, TCG, AGT, AGC	TCN, AGY
Glu/E	GAA, GAG	GAR	**Thr/T**	ACT, ACC, ACA, ACG	ACN
Gly/G	GGT, GGC, GGA, GGG	GGN	**Trp/W**	TGG	
His/H	CAT, CAC	CAY	**Tyr/Y**	TAT, TAC	TAY
Ile/I	ATT, ATC, ATA	ATH	**Val/V**	GTT, GTC, GTA, GTG	GTN
START	ATG		**STOP**	TAA, TGA, TAG	TAR, TRA

Table 4–1 Some Vital Statistics for the Human Genome

	HUMAN GENOME
DNA length	3.2×10^9 nucleotide pairs*
Number of genes	approximately 25,000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes**	more than 20,000
Percentage of DNA sequence in exons (protein coding sequences)	1.5%
Percentage of DNA in other highly conserved sequences***	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%

* The sequence of 2.85 billion nucleotides is known precisely (error rate of only about one in 100,000 nucleotides). The remaining DNA primarily consists of short highly repeated sequences that are tandemly repeated, with repeat numbers differing from one individual to the next.

** A pseudogene is a nucleotide sequence of DNA closely resembling that of a functional gene, but containing numerous mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene followed by the accumulation of damaging mutations in one copy.

*** Preserved functional regions; these include DNA encoding 5′ and 3′ UTRs (untranslated regions), structural and functional RNAs, and conserved protein-binding sites on the DNA.

Note: exon = expressed nucleotide sequence

Aminoacids

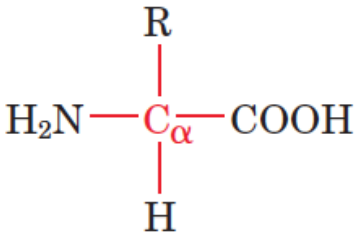


Figure 4-1 **General structural formula for α-amino acids.** There are 20 different R groups in the commonly occurring amino acids (Table 4-1).

*in chemistry, **zwitterions** act at the same time as acid and bases (while amphoteric compounds act either as acids or bases)*

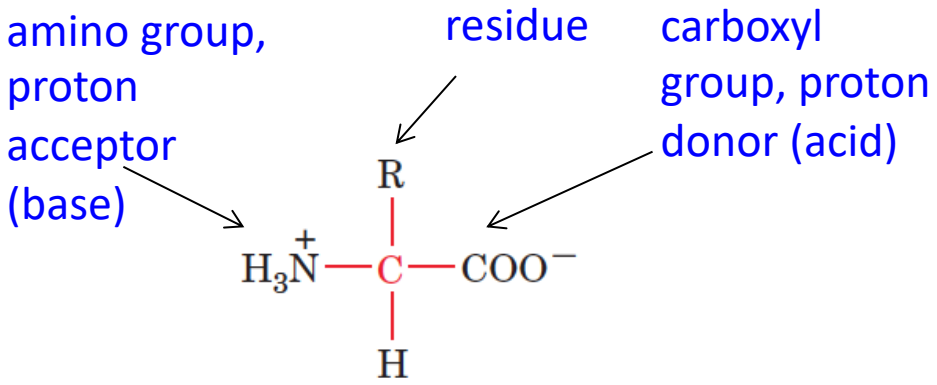


Figure 4-2 **Zwitterionic form of the α-amino acids that occurs at physiological pH values.**

Peptide bond

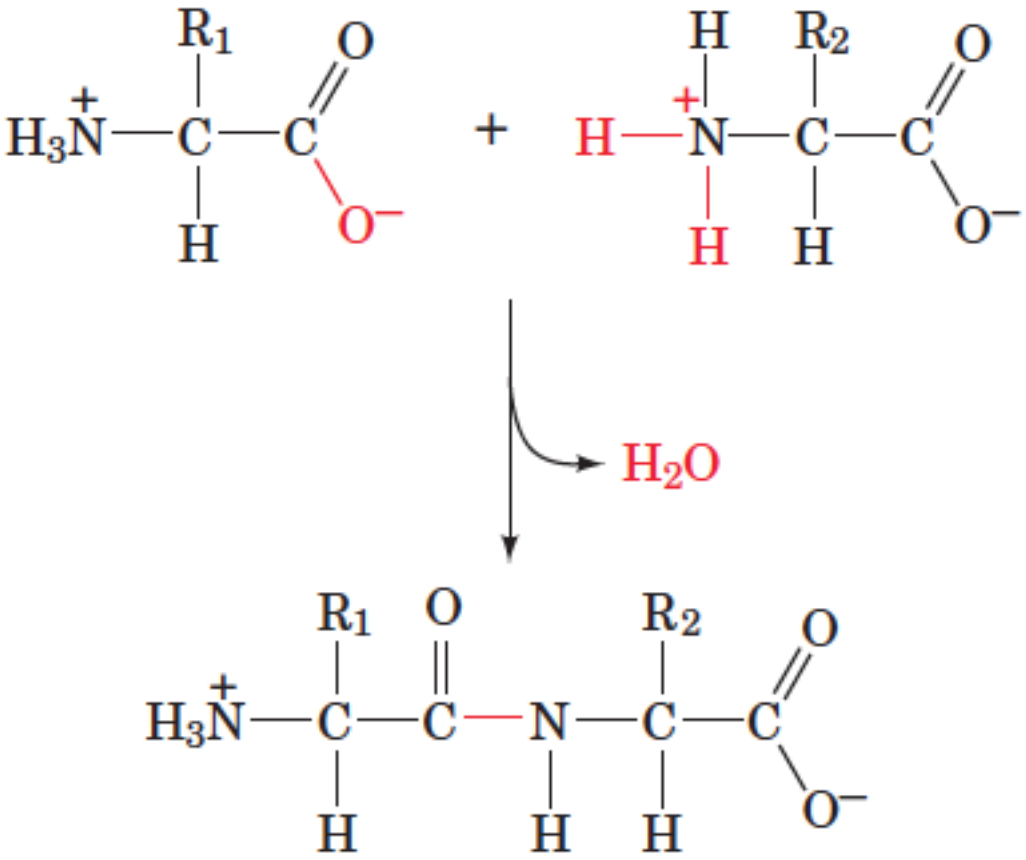


Figure 4-3 **Condensation of two α-amino acids to form a dipeptide.** The peptide bond is shown in red.

from D. Voet and J. G. Voet, "Biochemistry, 4th ed.", Wiley 2011

Proteins as polypeptide chains

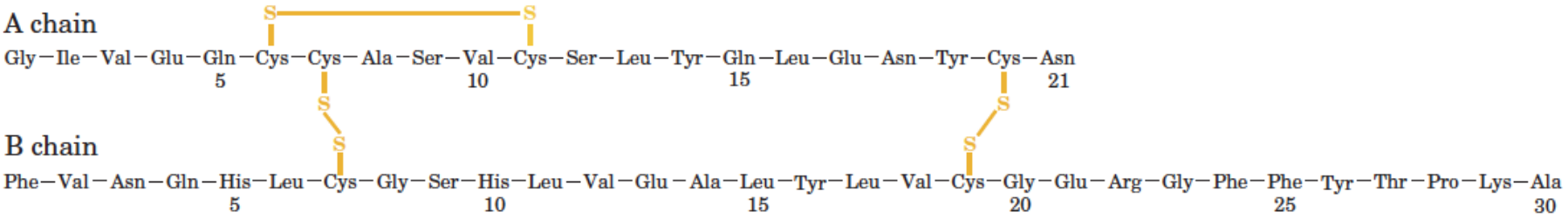


Figure 7-2 **Primary structure of bovine insulin.** Note the intrachain and interchain disulfide bond linkages.

from D. Voet and J. G. Voet, "Biochemistry, 4th ed.", Wiley 2011

Note that DNA codes for the linear chain, but post-transcriptional modifications produce the final chemical configuration of the protein.

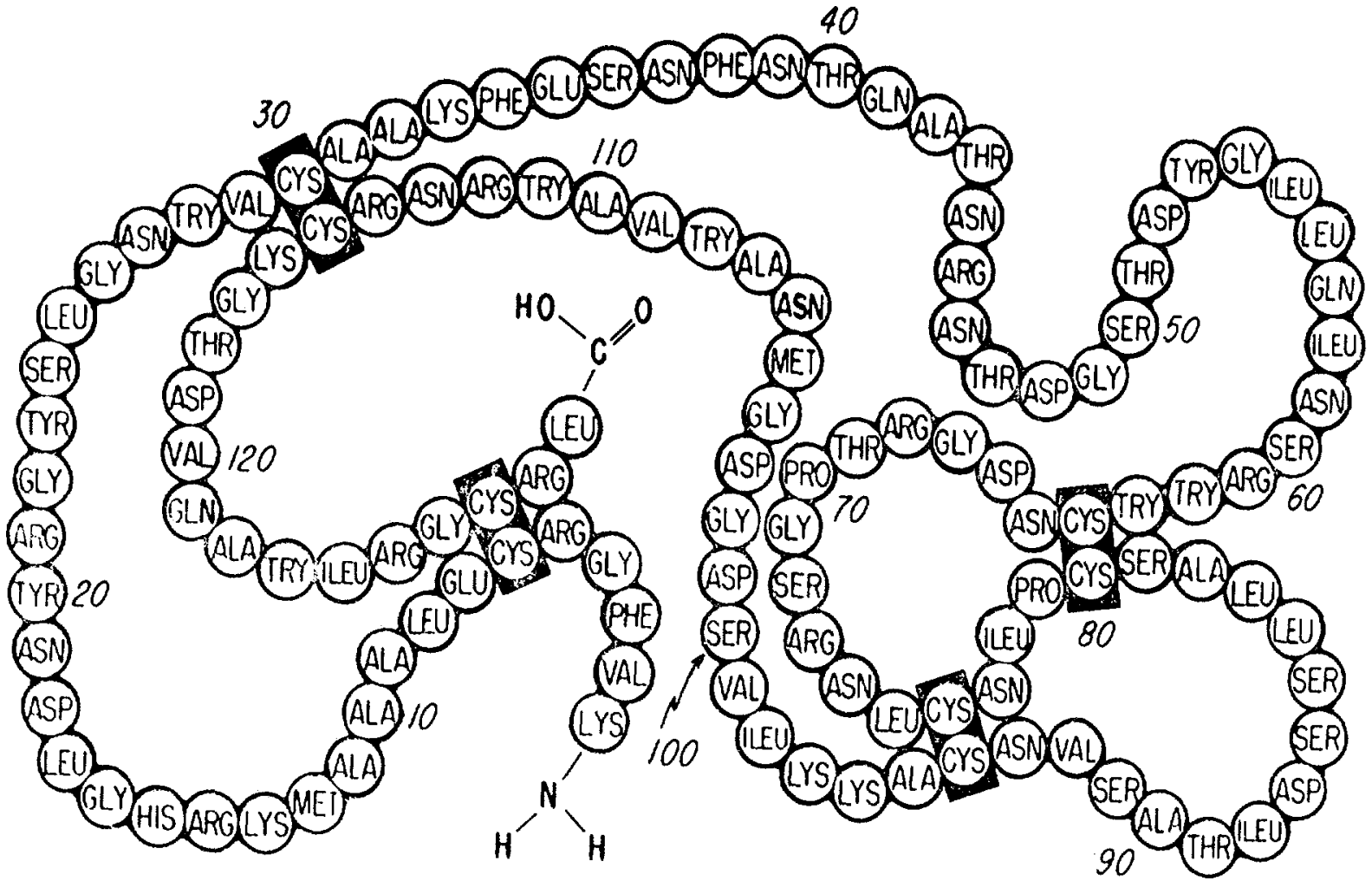
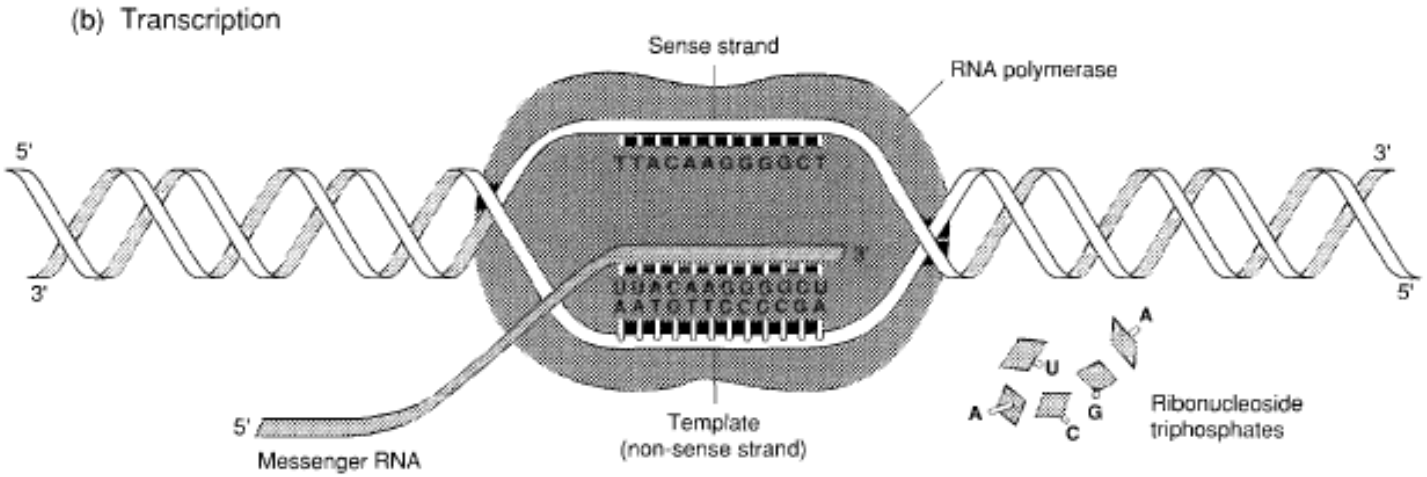
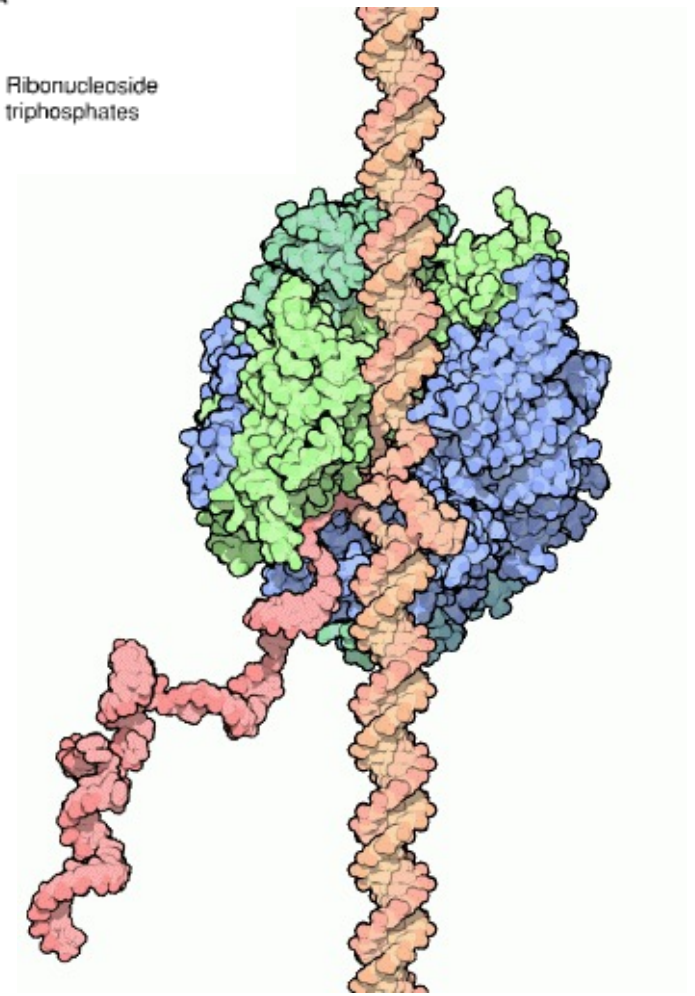


FIG. 1.—Amino acid sequence of hen egg-white lysozyme reproduced from Canfield and Liu, 1965.

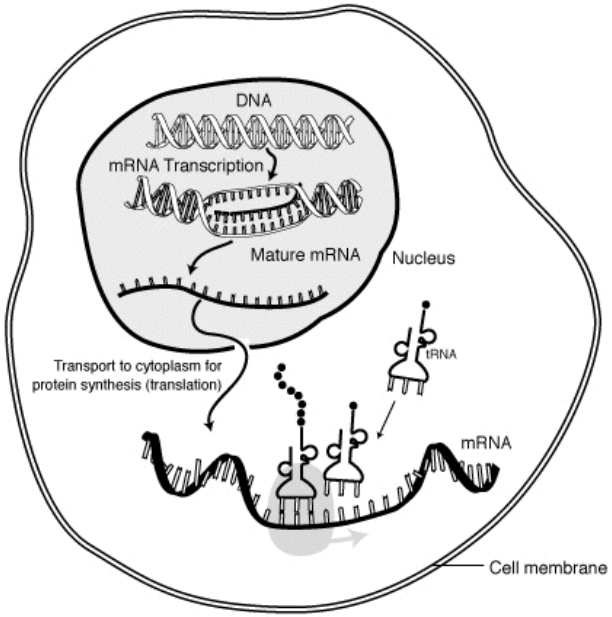


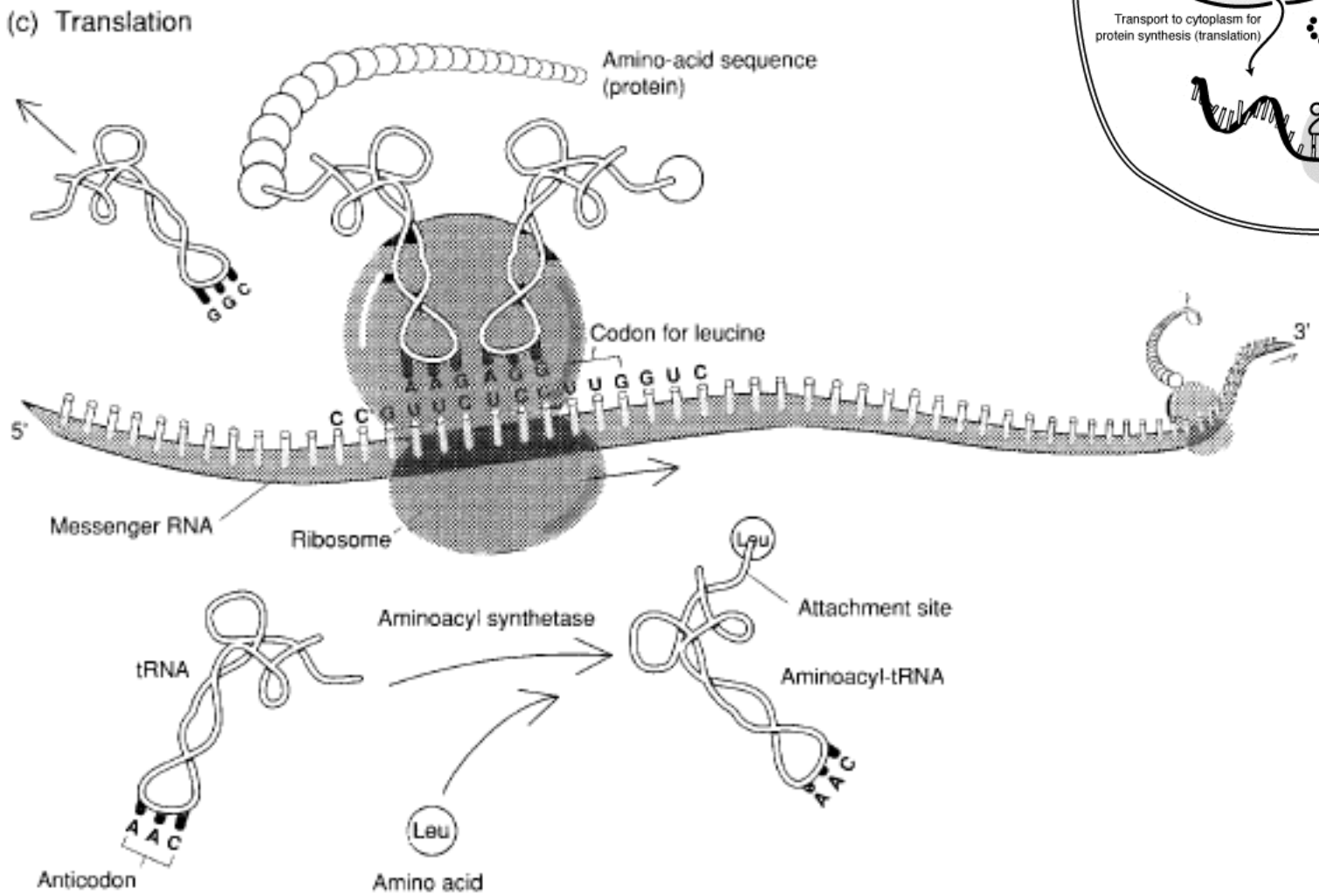
Proteins are encoded in DNA, but the message must first be transferred to messenger RNA (mRNA).

mRNA then brings this message to the ribosomes that act as true protein factories.



Transfer RNA (tRNA) selects the aminoacid that corresponds to a given codon and transports it to the ribosome. In this way the code transported by mRNA is sequentially translated into an aminoacid sequence and hence into a protein.





www.dnalc.org

A few numbers on ribosomes:

- there are approximately 10^6 ribosomes/cell ($2.5\ 10^6$ in liver cells)
- error rate about 10^{-5}–10^{-6}
- processing rate about 5–40 peptide bonds/s
- two units
  - small: molecular weight about 0.85 Mdalton, RNA about 1600 nucleotides, 21 proteins
  - large: molecular weight about 1.5 Mdalton, RNA about 3000 nucleotides, 34 proteins
- antibiotics often act against bacterial ribosomes (about 40% of all known antibiotics; molecular weight of antibiotics about 600–900 dalton; many work by steric hindrance)

The Nobel Prize in Chemistry 2009

Photo: U. Montan

Venkatraman Ramakrishnan

Prize share: 1/3

Photo: U. Montan

Thomas A. Steitz

Prize share: 1/3

Photo: U. Montan

Ada E. Yonath

Prize share: 1/3

The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath *"for studies of the structure and function of the ribosome"*.

Radiation damage to cellular structures

Radiation damages primarily DNA, the carrier of genetic information, and **we focus here primarily on the structure of DNA**.

However **other structures are also damaged by radiation** and in particular

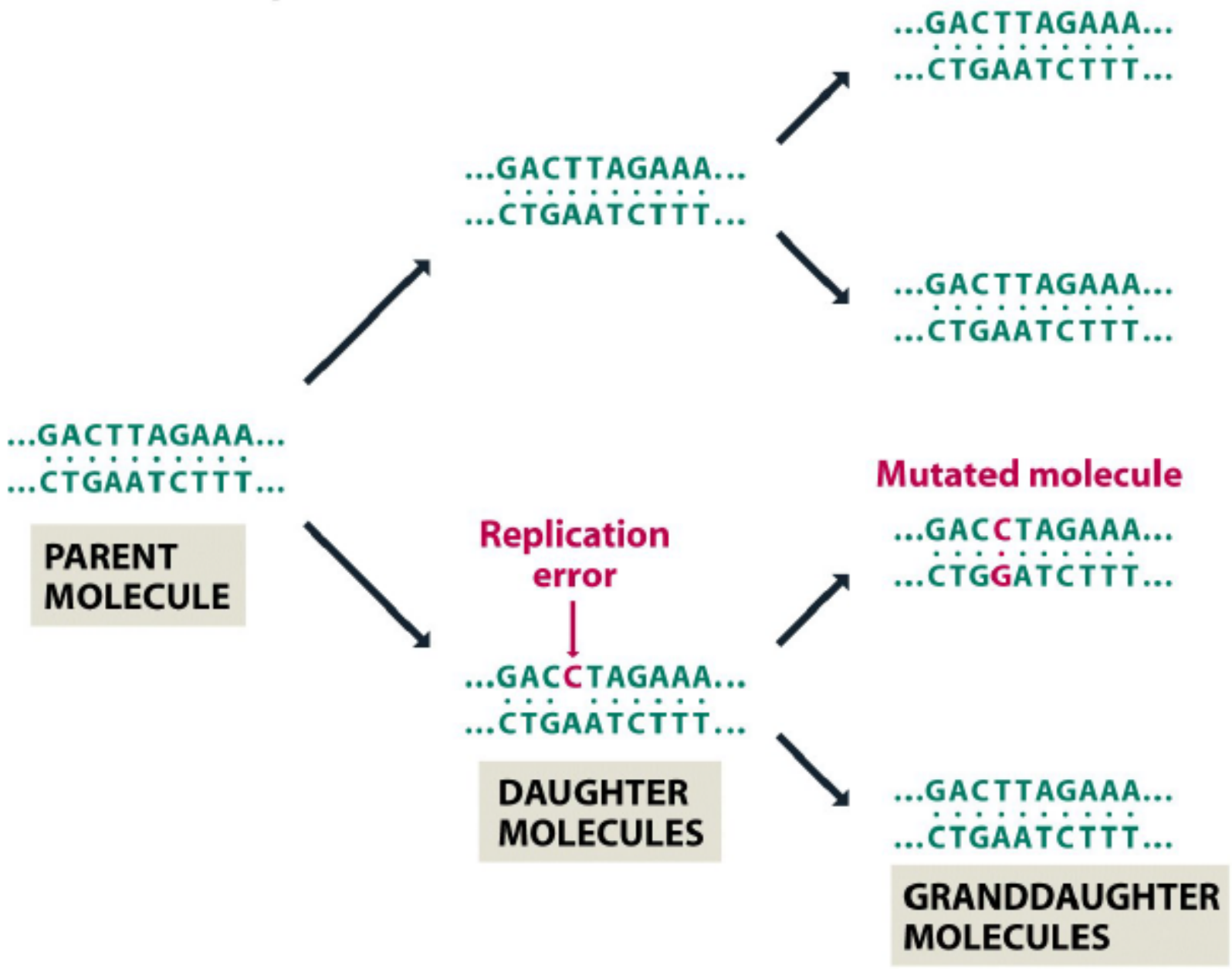
- membranes
- mitochondrial DNA
- proteins

DNA damage

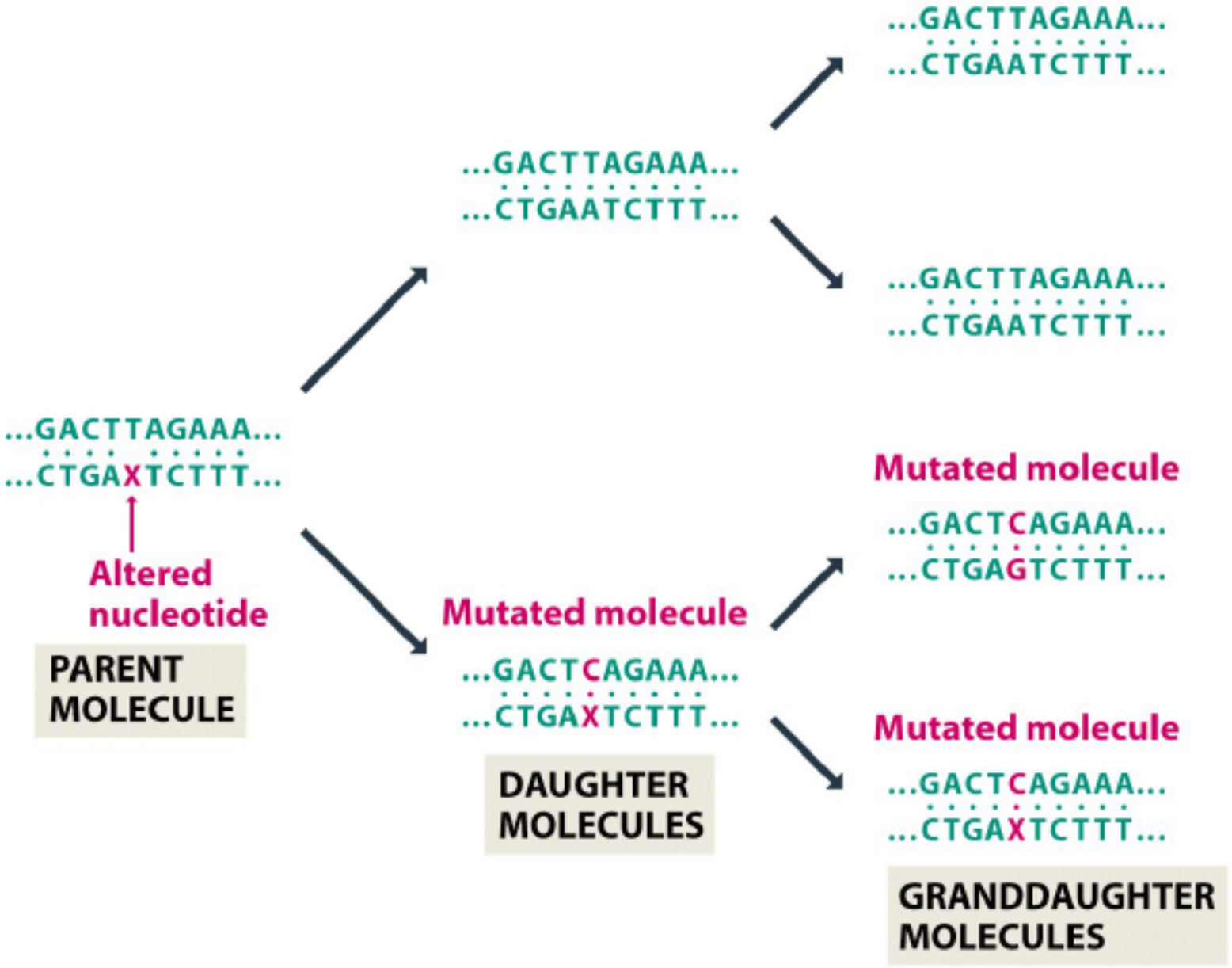
DNA can be damaged by

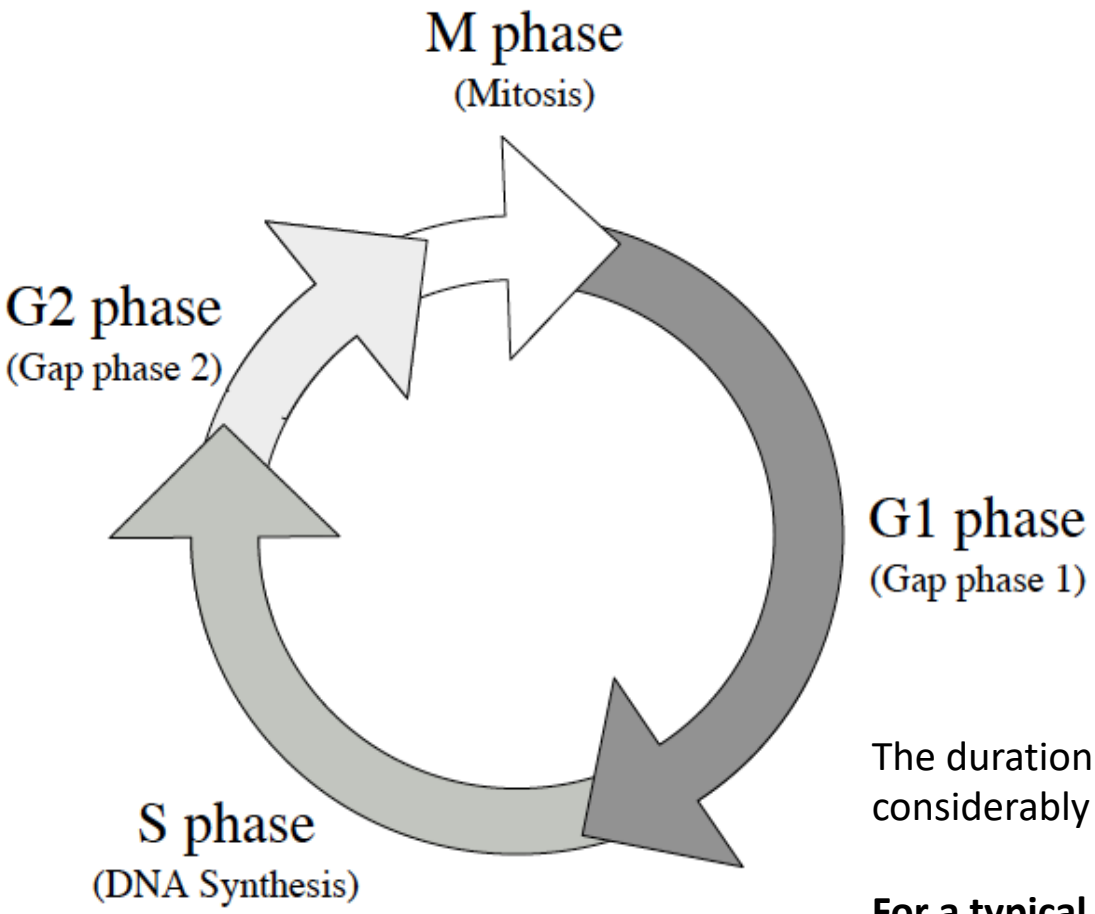
- Genotoxic chemicals (exogenous damage)
- Radiation (exogenous damage)
- Reactive species from cell metabolism (endogenous damage)

An error in replication



One possible effect of a mutagen

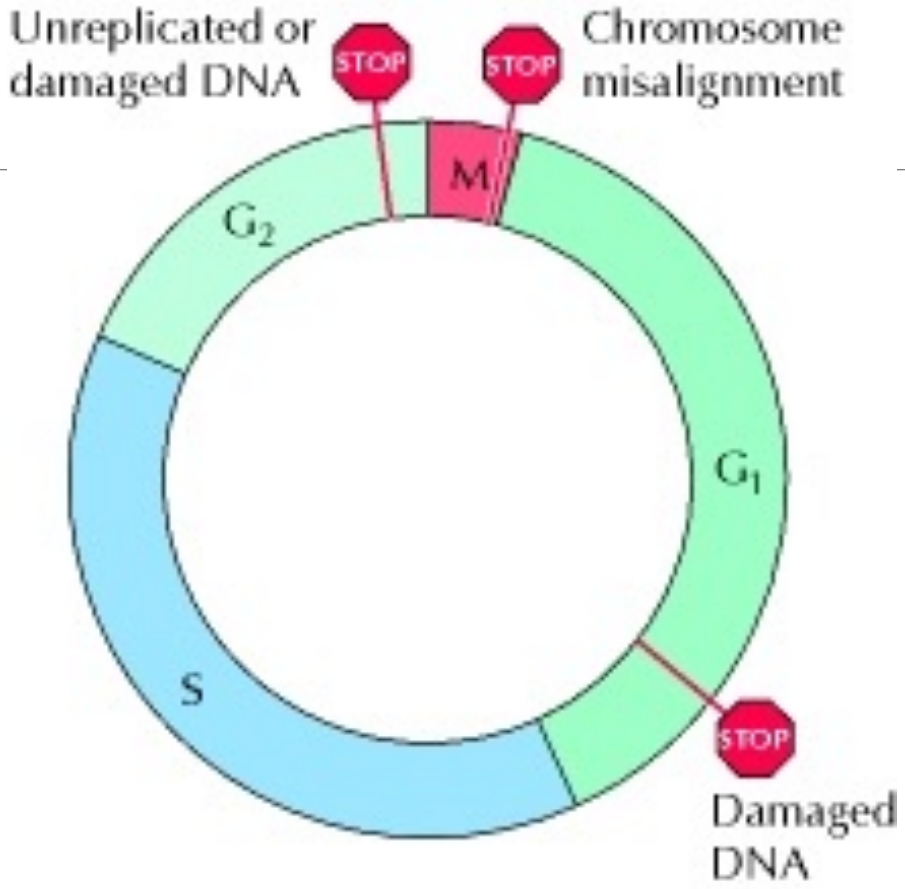




The duration of these cell cycle phases varies considerably in different kinds of cells.

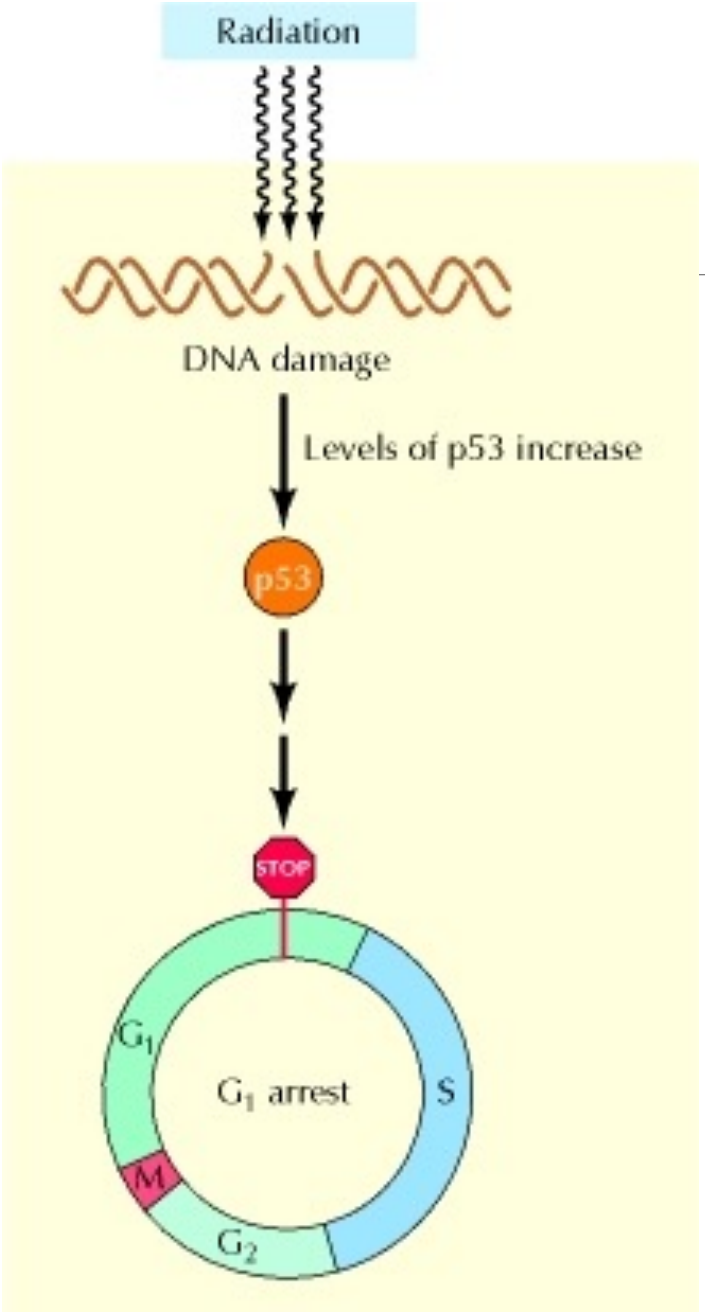
For a typical rapidly proliferating human cell with a total cycle time of 24 hours, the G1 phase might last about 11 hours, S phase about 8 hours, G2 about 4 hours, and M about 1 hour.

(source: Geoffrey M. Cooper e Robert E. Hausman: "The Cell: A Molecular Approach. 5th ed.", Sinauer Associates 2009.)



Cell cycle checkpoints.

(source: Geoffrey M. Cooper e Robert E. Hausman: "The Cell: A Molecular Approach. 5th ed.", Sinauer Associates 2009.)



DNA damage, such as that resulting from irradiation, leads to rapid increases in p53 levels. The protein p53 then signals cell cycle arrest at the G1 checkpoint.

(source: Geoffrey M. Cooper e Robert E. Hausman: "The Cell: A Molecular Approach. 5th ed.", Sinauer Associates 2009.)

Direct repair

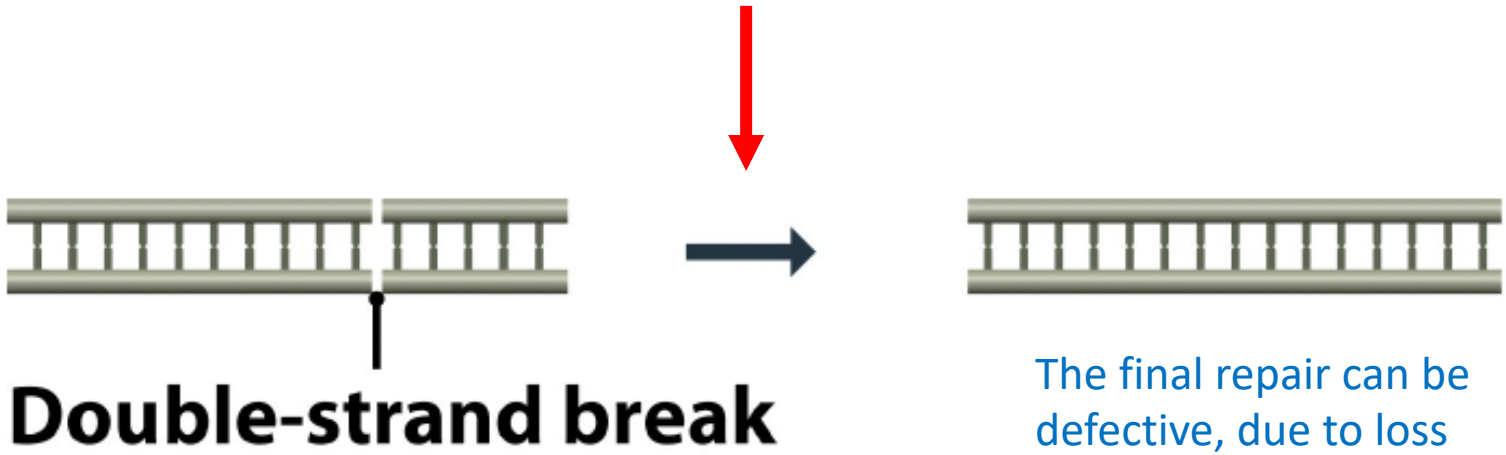
Figure 16.20a *Genomes 3* (© Garland Science 2007)

Excision repair

Mismatch repair

Double strand breaks can be repaired in two main ways

In different conditions, the repair process can follow two main paths: **nonhomologous end joining** or **homologous recombination**



The final repair can be defective, due to loss of information

Figure 16.20d *Genomes 3* (© Garland Science 2007)

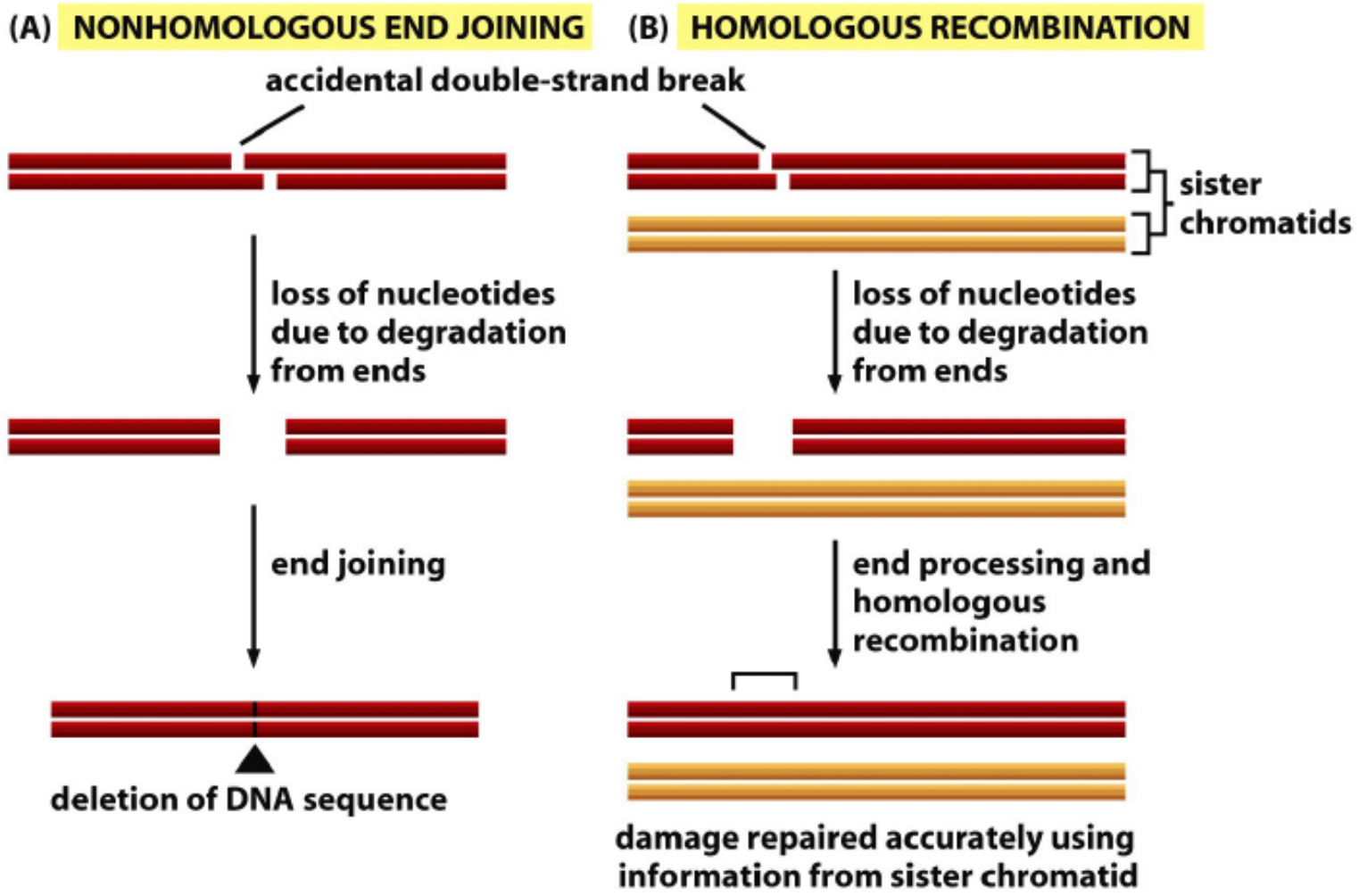


Figure 5-51 *Molecular Biology of the Cell* (© Garland Science 2008)

Mutations

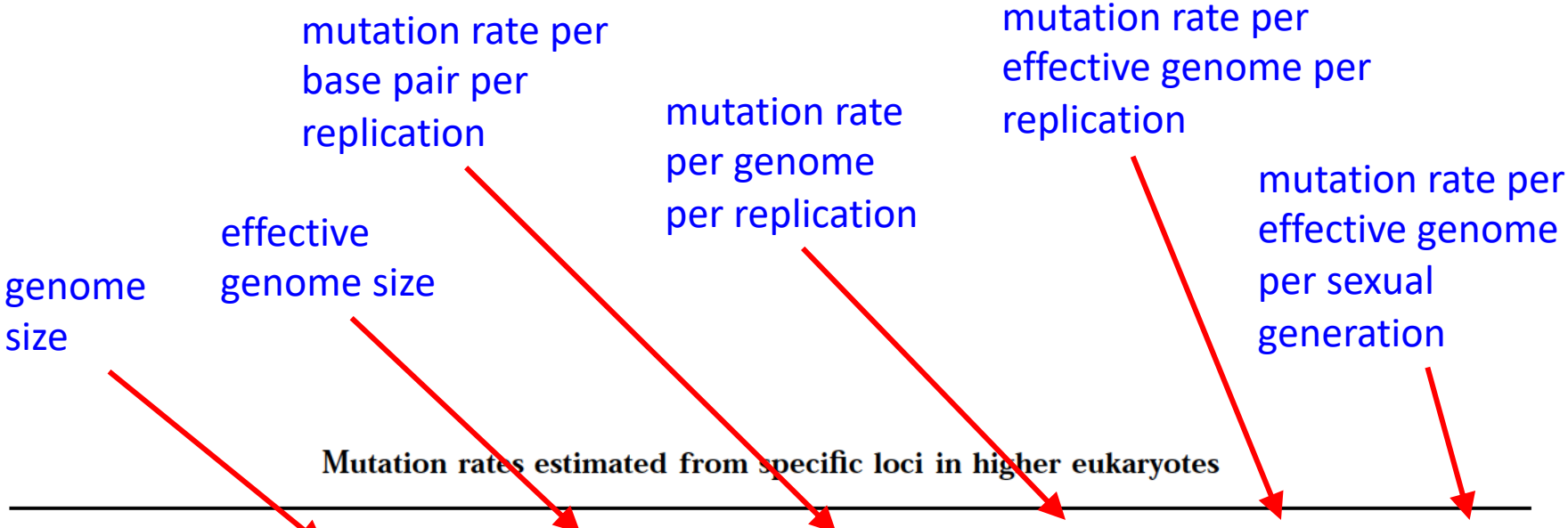
Sometimes errors in DNA duplication or repair occur, giving rise to new nucleotide sequences: **these errors are called mutations.**

Mutations can occur both in somatic or germ cells, however mutations in somatic cells are not inherited and they can be neglected from an evolutionary point of view.

However, **mutations in somatic cells are also important because they can lead to disease and death**.

Estimates of mutation rates

(very difficult, only few estimates exist... this one is from Drake et al., "Rates of Spontaneous Mutation", Genetics 148 (1998) 1667)



Mutation rates estimated from specific loci in higher eukaryotes

Organism	G	G_e	μ_b	μ_g	μ_{eg}	μ_{egs}
C. elegans	8.0×10^7	1.8×10^7	2.3×10^{-10}	0.018	0.004	0.036
Drosophila	1.7×10^8	1.6×10^7	3.4×10^{-10}	0.058	0.005	0.14
Mouse	2.7×10^9	8.0×10^7	1.8×10^{-10}	0.49	0.014	0.9
Human	3.2×10^9	8.0×10^7	5.0×10^{-11}	0.16	0.004	1.6

These are all likely to be underestimates, because not all kinds of mutations are included, particularly those with minor, inconspicuous effects.

Radiation damage to cellular structures

In general, to destroy cell function in non-proliferating cells a typical dose of 100 Gy is required, while to destroy proliferative cell capacity requires typically only few Gy's.

When directly ionizing radiation is absorbed in biological material, the damage to the cell may occur in one of two mechanisms:

- **Direct**
- **Indirect**

Here we focus mainly on the damage to DNA, however the damage to proteins and membranes is also important.

Direct and indirect action

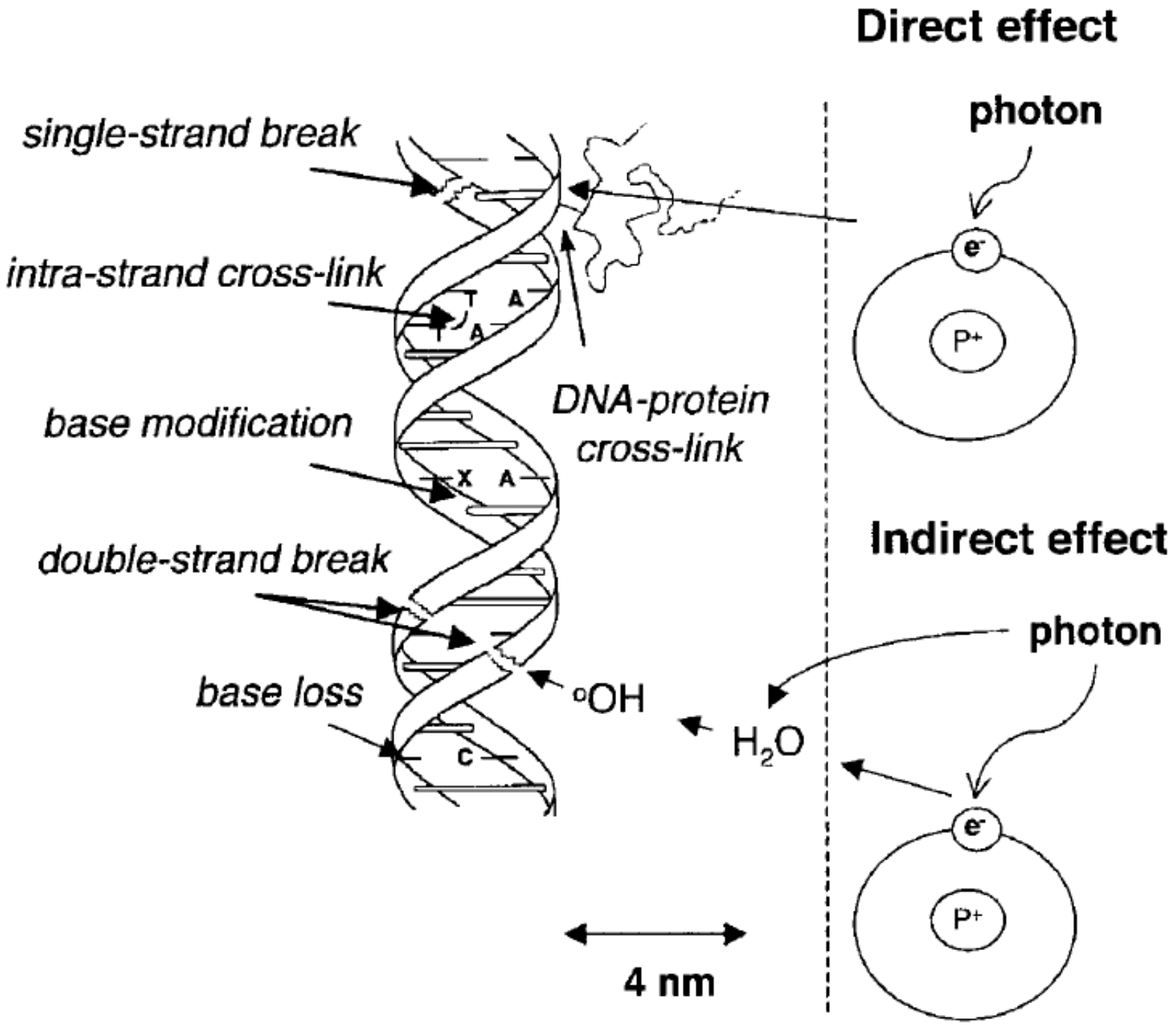
In direct action, the radiation interacts directly with a critical target in the cell, by breaking chemical bonds.

Charged particles can do this without intermediate steps.

Neutral particles (e.g., photons) must first be absorbed, and the damage is caused by the charged particles that are emitted after the primary interaction (e.g., the electrons and positrons in the electromagnetic cascade produced by a high-energy photon)

In indirect action, the ROS produced by the incoming radiation break the chemical bonds in the affected targets

Indirect damage accounts for as much as 2/3 of the total radiation damage.


Direct effect
photon
single-strand break
intra-strand cross-link
base modification
DNA-protein cross-link
double-strand break
base loss
Indirect effect
photon
OH
H2O
4 nm

ROS action

We have already met several examples of ROS such as the hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hydroperoxyl radical ($HO_2^{\cdot}$) and singlet oxygen ($^1O_2$).

In cells, normal biochemical and physiological processes such as the β-oxidation of fatty acids in the peroxisomes, the electron transport chain in the mitochondria and ATP production by cells are endogenous source of ROS.

Being able to produce ROS, ionizing radiation is an exogenous source of ROS.

Very reactive species such as the $^{\cdot}OH$ radicals react in a restricted radius from their site of creation with neighboring molecules such as proteins, lipids and nucleic acid by chemical modification.

ROS action /ctd.

Other radiolytic species like H_2O_2 are much less reactive.

However, in the presence of transition metals such as iron or copper, H_2O_2 can generate an $^{\cdot}OH$ radical by the *Fenton reaction*:

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$
$$H_2O_2 + Cu^{+} \rightarrow {}^{\bullet}OH + OH^- + Cu^{2+}$$

Oxidative stress

In indirect action the radiation interacts with other molecules and atoms (mainly water, since about 80% of a cell is composed of water) within the cell to produce free radicals, which can, through diffusion in the cell, damage the critical target within the cell.

This kind of damage is activated by metabolites that occur naturally inside cells, or by ROS.

Globally this is called **oxidative stress**, it exists even in absence of radiation or externally introduces oxidizing chemicals, and the damage associated to self-produced chemicals is called **endogenous damage**.

Indirect action can be modified by chemical sensitizers or radiation protectors.

Oxidative stress /ctd.

There are several important defense mechanisms against oxidative stress.

One of them involves **superoxide dismutase** (SOD) which converts the superoxide anion O_2^- into oxygen (O_2) or hydrogen peroxide (H_2O_2)

Another one is **catalase**, which converts the still dangerous hydrogen peroxide into water and oxygen (O_2).

Still another class of enzymes are the **peroxiredoxins**, which also convert hydrogen peroxide into water and oxygen (O_2).

Formation and Elimination of Reactive Oxygen Species (ROS)

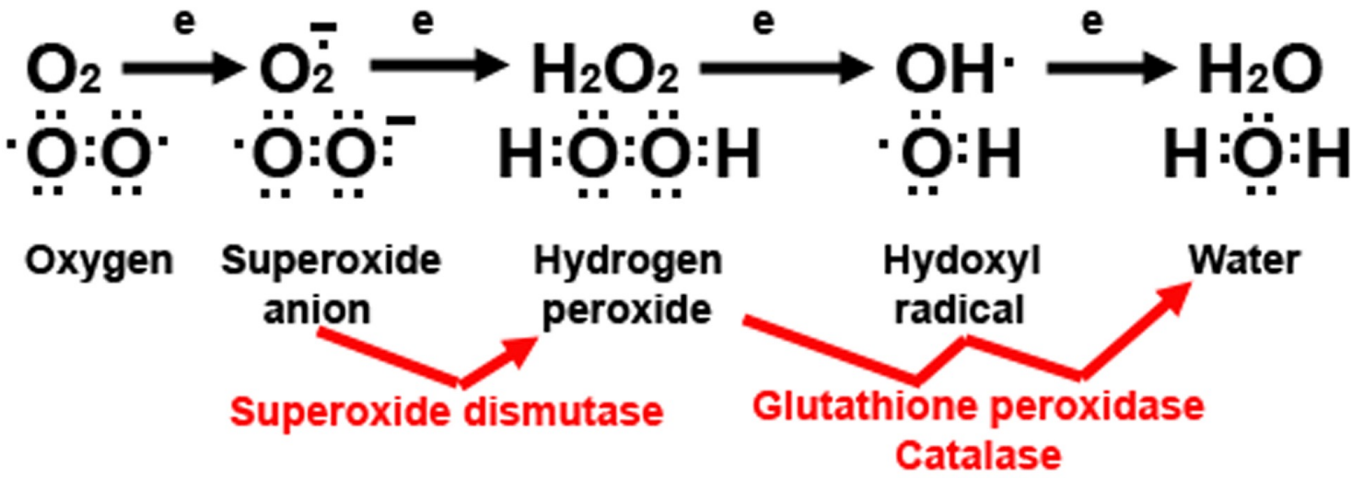
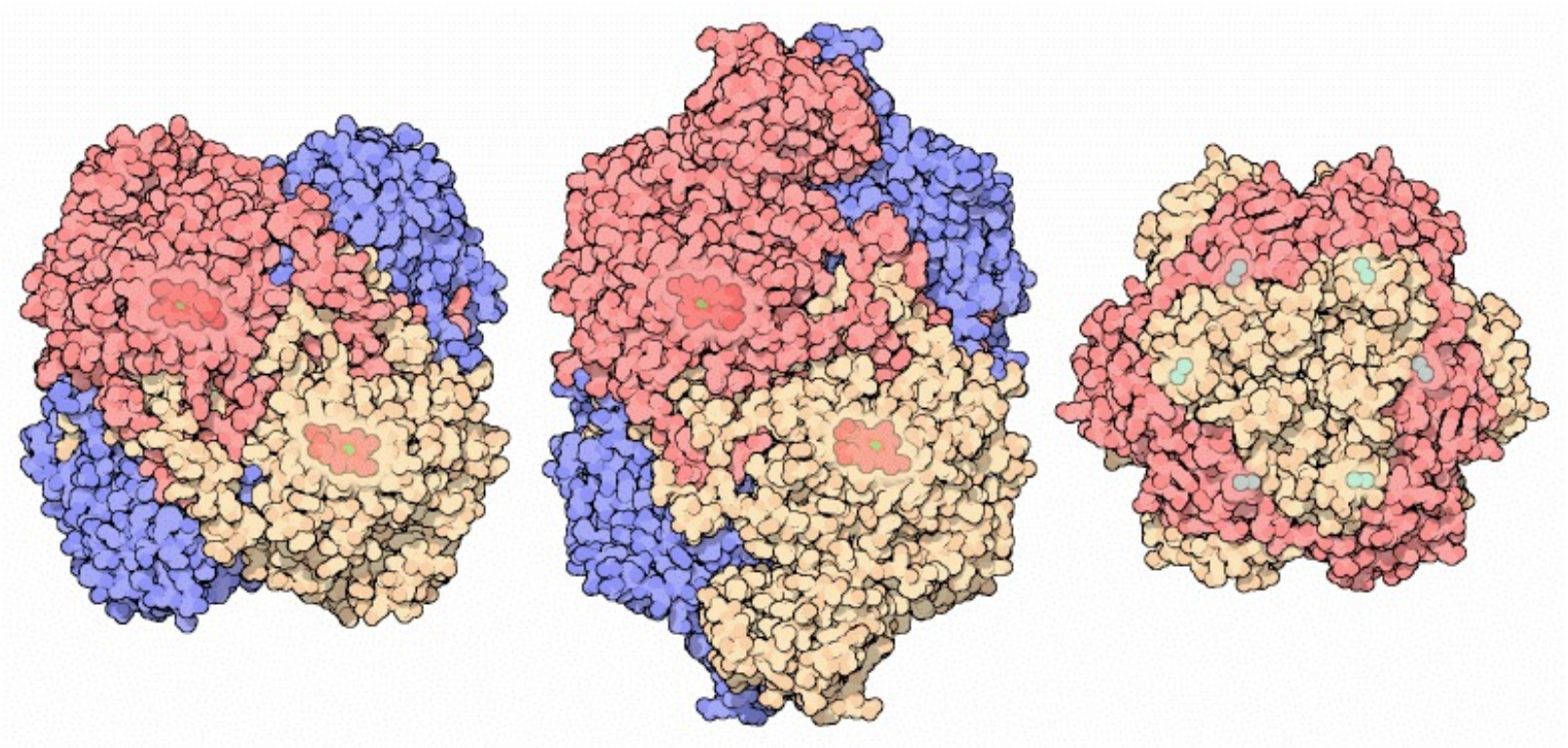


Diagram of reactive oxygen (ROS) formation and elimination. Oxygen (O_2) plays a major role in the formation of ROS because O_2 has unpaired electrons (represented by single dots). When O_2 picks up an electron, it becomes superoxide, an extremely reactive anion. Superoxide dismutase catalyzes the dismutation reaction of superoxide to hydrogen peroxide, which is further catalyzed to the highly reactive hydroxyl radical and ultimately to water by glutathione peroxidase and catalase enzymes. Superoxide, hydrogen peroxide, and hydroxyl radicals are considered to be ROS.

There are several kinds of catalase, and they are very efficient: one molecule of catalase can convert hydrogen peroxide into water at the rate of ~10^6 molecules/s.



Human red blood cells catalase (iron-based)

Bacterial iron-based catalase

Bacterial manganese-based catalase

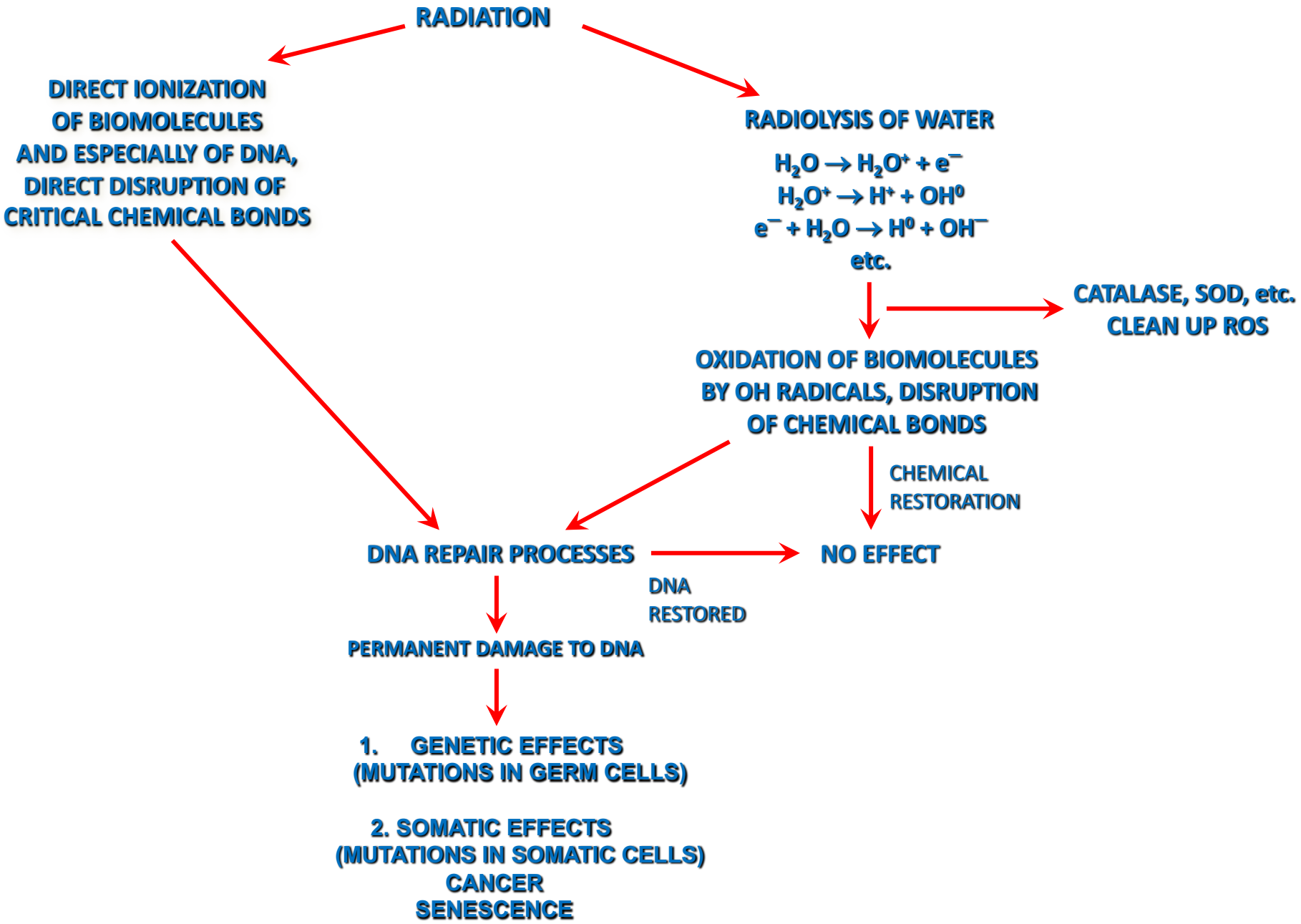
Mutations or absence of these proteins lead to several important pathologies, for example:

SOD:

- a mutation of SOD has a role in familial **amyotrophic lateral sclerosis (ASL)**
- in mice, inactivation of SOD2 causes perinatal lethality and inactivation of SOD1 causes hepatocellular carcinoma
- diminished activity is linked to hypertension and to pulmonary diseases
- SOD has powerful pharmacological properties, among them it is an antiinflammatory

Catalase:

- catalase deficiency may increase the likelihood of developing type 2 diabetes
- catalase deficiency in hair follicles may play a role in hair graying


RADIATION
DIRECT IONIZATION OF BIOMOLECULES AND ESPECIALLY OF DNA, DIRECT DISRUPTION OF CRITICAL CHEMICAL BONDS
RADIOLYSIS OF WATER
$H_2O \rightarrow H_2O^+ + e^-$
$H_2O^+ \rightarrow H^+ + OH^0$
$e^- + H_2O \rightarrow H^0 + OH^-$
etc.
CATALASE, SOD, etc. CLEAN UP ROS
OXIDATION OF BIOMOLECULES BY OH RADICALS, DISRUPTION OF CHEMICAL BONDS
CHEMICAL RESTORATION
DNA REPAIR PROCESSES
NO EFFECT
DNA RESTORED
PERMANENT DAMAGE TO DNA
1. GENETIC EFFECTS (MUTATIONS IN GERM CELLS)
2. SOMATIC EFFECTS (MUTATIONS IN SOMATIC CELLS) CANCER SENESCENCE